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ERRATA

p. 1, l. 1, for "de Wildeman" read "De Wildeman."
1, l. 14, for "similarily" read "similarly."
p. 83, l. 13, for "**Hammer**" read "**Hamner.**"
p. 86, l. 11, 13, 16, for "**Moxon**" read "**Maxon.**"
p. 169, l. 27, for "*Pottiae*" read "*Pottia.*"
p. 239, l. 22, delete the reference to "**C(arter)**"
l. 24, for "pyrenomycestes" read "Pyrenomycetes," and before "Hill" insert "Tile."
p. 240, l. 10, for "Monographe du genre *Cistes*" read "Monographie du genre *Cistus.*"
p. 241, l. 24, for "indolelacetie" read "indolylacetie."
p. 245, l. 42, for "*Helianthemun*" read "*Helianthemum.*"
p. 246, l. 38, for "*Paeania*" read "*Paeonia.*"
p. 248, l. 18, for "*Fuscarium*" read "*Fusarium.*"
p. 367, Table 3, Transpose "orthodox" and "unorthodox."
p. 380, l. 5, omit comma after "here."
p. 412, l. 17, for "**Heinreicher**" read "**Heinricher.**"
p. 427, l. 14, for "*Preusii*" read "*Preussii.*"
p. 585, l. 23, for "*linarifolius*" read "*linariifolius.*"
l. 24, for "*rotunifolia*" read "*rotundifolia.*"
p. 589, l. 10, for "*Goodyeria*" read "*Goodyera.*"
l. 12, for "*cryptolepsis*" read "*cryptolepis.*"

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VOLUME 67 JANUARY · 1940 NUMBER 1

The Cytology of Endochytrium operculatum (de Wildeman) Karling in Relation to its Development and Organization

ARTHUR B. HILLEGAS

(WITH PLATES 1–7)

INTRODUCTION

As now recognized by most mycologists, the family Rhizidiaceae is the largest of the chytrid groups; it is reported to include 31 genera and about 198 species. Whether or not it constitutes a natural phylogenetic group or several separate families is at present uncertain. The family as a whole is characterized by a monocentric thallus which consists of an incipient zoosporangium or resting spore and an absorbing or rhizoidal system of varying complexity and size. In most cases resting spores are unknown or formed asexually similarily to the zoosporangia, but sexual reproduction has been reported in about 10 genera and 21 species, and exhibits great variability. Fusion of the protoplasts of equal and unequal thalli through pores, rhizoids, and conjugation tubes; fusion of motile isogametes, and a small female thallus with a motile male gamete or vice versa have been described in the literature. Accordingly, on the basis of variations in sexuality, few of the genera in this family seem to be closely related.

In spite of its size and significance in the phylogeny of the Chytridiales, and the wide variations in sexuality it exhibits, the family Rhizidiaceae has attracted very little attention from cytologists, and so far only three or possibly four species, *Polyphagus Euglenae*, *Zygorhizidium Willei*, *Sporophlyctis rostrata* and *Rhizophidium Beauchampi* have been studied from fixed and stained material.

The cytological problems presented by the Rhizidiaceae are numerous and varied. Before we can arrive at definite and final conclusions as to

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the phylogeny and the relationships of the family, the cytology of the varied types of sexual reproduction, cell and nuclear fusions, meiosis, and their relations to the alternation of monoploid and diploid generations must be understood. So far it is not known whether the respective gametes are derived from the same or different thalli and gametangia, nor at what stage in the life cycle meiosis occurs, and little is known about the details of nuclear division, cleavage, sporogenesis, and the structure of the swarmspores in the group as a whole. One of the most baffling and interesting problems presented by this group is the cause of the constancy of the monocentric type of thallus. It has been suggested by Karling (1937b) that this is associated with, or perhaps determined by, the localization of the nuclei in the incipient zoosporangia and resting spores during the development of the thallus. A thorough study of the relation of nuclear distribution to the development of the thallus might thus throw some light on the causes of the characteristic monocentric type of organization in the Rhizidiaceae.

Endochytrium operculatum has been chosen for the study of some of the problems noted above because it is a typical, intramatrical representative of the Rhizidiaceae, and because of its large size, availability and abundance throughout the year. The identity and possible relationships of this species have been fully discussed by Karling (1937a) so that they need not be further considered here.

MATERIALS AND METHODS

Endochytrium operculatum grows intramatrically in cells of *Nitella* and *Cladophora*. The material used for this study was obtained at Columbia University by placing bleached and cooked filaments of these algae in greenhouse tanks previously infected with *Endochytrium*. These filaments were held in small cheesecloth or bobinet bags as employed by Karling (1935) in his study of *Cladochytrium replicatum*. Usually a heavy infection of *Endochytrium* occurred in six to ten days. The infected material was brought into the laboratory and washed in distilled water to remove bacteria and protozoa. It was then transferred to fresh spring water containing bleached and sterilized *Cladphora* filaments. About every ten days the cultures were washed and transferred to culture dishes containing fresh water and freshly prepared *Cladophora* filaments. In this way was maintained a stock culture of the fungus from which sub-cultures could be started. Sterile charcoal water was sometimes used instead of spring water, but it was found to check vegetative development and induce the formation of resting spores. In order to facilitate seeing the fungus

within, the *Nitella* filaments were first boiled in alcohol to remove the chlorophyl as done by Karling (1937a) in his studies of *E. operculatum.*

For observation of the living chytrid, *Cladophora* was found to be more convenient, since it may be kept dried in the laboratory for use at any time. The filaments of *Cladophora* growing out-of-doors are usually covered with such a growth of diatoms and other epiphytes that the intramatrical chytrid is obscured. To remove this overgrowth from the algal filaments, to soften the wall, and to make the protoplasm more homogeneous, the dried algae were boiled in 10% KOH for 15 minutes and then thoroughly washed in water. There seems to be little difference in the degree of infection between the material treated by hydroxide and the untreated material. Another method employed in removing the diatoms was to place the filaments in 15% hydrofluoric acid for three days, and then wash in water for several hours. Although in this case the *Cladophora* cells became heavily infected with *E. operculatum*, its subsequent rapid deterioration made such material unsuitable for study.

For sectioning and staining, infected internodes of *Nitella* were used exclusively, since the cell wall of *Cladophora* is difficult to section. A variety of fixatives was used: Allen and Wilson's modification of Bouin's, Merkel's, Carnoy's, absolute alcohol, chrom-acetic, Feulgen's, and Flemming's strong, medium, and weak solutions in various dilutions. Allen and Wilson's fixative in normal and one-half strength, Flemming's medium in one-fourth strength, and Flemming's weak solution diluted with distilled water to one-half strength were found to give the best results and were regularly employed. The other fixatives were used only for special purposes. Forty-eight hour fixing was found to give more satisfactory results than shorter periods.

Whole mounts, for the study of nuclear distribution in relation to organization, were prepared by fixing and washing *Cladophora* and *Nitella* in the usual manner, and then staining them *in toto* in vials with Heidenhain's iron-haematoxylin. The filaments were dehydrated and removed to a hanging-drop slide where they were counter-stained with alcoholic Orange G., rinsed with absolute alcohol, and cleared in xylol. Next, the material was placed in xylol on a slide, separated, and covered with balsam. Permanent mounts were also made in lacto-phenol containing a mixture of methyl blue and acid fuschin according to Maneval's formula (1936). This was a very satisfactory way of demonstrating the presence of nuclei in developing sporangia and resting spores.

Flemming's triple stain, Feulgen's, and Heidenhain's iron haematoxylin were relied upon for details of nuclear structure. Janus green

which is claimed to be specific for chondriosomes was used as an intravitam stain in dilutions of 1:100,000, 1:250,000, and 1:500,000.

To determine the composition of the large amount of highly refractive substance found in all stages of development of the fungus, ether, xylol, chloroform, acetic acid, and the fat stains Sudan III and Scarlach R (Sudan IV), Nile blue sulfate, and osmic acid were used. In addition, two tests for glycogen were made: the iodine test with iodine-potassium iodide, and Best's carmine method, according to Galigher (1934). The cell walls were tested for cellulose with iodine-potassium iodide followed by sulfuric acid and zinc chloriodide, and for chitin by hydrolysis with potassium hydroxide to chitosan using the method described by Rawlins (1933).

Zoospores and their germination stages were studied from fixed and stained preparations. If germination stages were desired, the slide was smeared with a drop of agar and dried by being passed through a flame two or three times. *Cladophora* filaments containing sporangia about to discharge their zoospores were placed in a drop of distilled water on the slide and the slide placed in a moist chamber, until the zoospores had been discharged or germination had occurred. For fixation the slide was inverted over the mouth of a bottle containing 2% osmic acid for 30 seconds, dried, and passed twice through an alcohol flame to fix the zoospores or germination stages to the slide. The material was stained in acid fuchsin—cotton blue in lacto-phenol, either directly or after fixation for two hours in Allen and Wilson's or Flemming's weak solution. The stain used was either Heidenhain's haematoxylin followed by alcoholic Orange G., or gentian violet after the method of Cotner (1930a).

OBSERVATIONS

Since the structure and development of *Endochytrium operculatum* (de Wildeman) Karling has been described from living material by de Wildeman (1895), Sparrow (1933), and Karling (1937a), it would be superfluous to repeat their observations. However, in order to compare the stages in living material with the corresponding stages in fixed and stained material, it will be necessary to deal briefly with the developmental stages in both.

Structure of the Zoospore

The living zoospore is hyaline, spherical to oval in shape, 3.9μ to 5.4μ in diameter with a single posterior cilium about five times as long as

the diameter of the zoospore body. The active swimming period of the zoospore varies from several minutes to a few hours, with occasional interruptions of quiescent and amoeboid periods during which the zoospores creep about on the substratum for a few seconds or minutes, then round up again and swim away. In exceptional cases, zoospores discharged into the host cell remain active for as long as six hours. The most prominent structure of the zoospore is the large, highly refractive globule, 1.57–2.4μ in diameter, whose position relative to the cilium does not appear to be fixed. It may lie in the anterior position, as in fig. 1, near the membrane at the side, as in fig. 2, or near the posterior end of the zoospore. Thus if the globule is oriented toward the cover slip, it may appear to occupy the center of the zoospore, as in fig. 3. The globule appears quite fluid and when the zoospore creeps through a tight place under the cover glass, the globule may undergo changes in shape as shown in figure 4. Its composition is apparently very complex, but it usually gives much the same reaction as oil. It is usually dissolved by xylol in the fixation and staining of material, leaving a more or less clear space in the cytoplasm at the anterior end of the zoospore as in figure 5, or in whatever region it occupied at the time of fixation. Figure 6 shows a fixed and stained zoospore with a lighter region in the anterior part of the nuclear cap which resembles the space left in the dissolution, of the refringent globule. If such is the case, it is obvious that the globule may sometimes lie in the substance which makes up this cap.

Closely associated with the refringent globule in the living material is another body of a rather indefinite outline and structure, which may be the nucleus and nuclear cap which are so conspicuous in fixed and stained material. In addition to these structures there may be a small granule which moves about in the cytoplasm and occasionally one or more granules in the cytoplasm as in figure 1.

The fixed and stained zoospore is quite different in appearance from the living zoospore. The refringent globule, as such, is no longer present, and the most conspicuous structure is the clear, spherical nucleus partly surrounded by a densely staining mass which constitutes the so-called extra-nuclear cap as is shown in figures 5 and 6. Since the nucleus is usually less than 2μ in diameter and is surrounded by such a large amount of extra-nuclear material it is difficult to see its internal structure. As is shown in figures 5–10, it is usually spherical in shape, comparatively transparent, and may be identified by the small, densely stainable, somewhat flattened, disc-shaped nucleolus. This structure is particularly evident near the zoospore wall in figure 10. As is shown in

this figure and in figure 7, a second densely stainable body may be present, but it appears to be outside of the nuclear membrane and constitutes a part of the nuclear cap. Besides the nucleole little can be seen, and I have been unable to observe a definite nuclear reticulum.

The nuclear cap stains deeply with haematoxylin, safranin and gentian violet but, like the nucleole, does not stand out very sharply in shape and color when fixed and stained by the Feulgen technique. It varies considerably in size and shape as figures 5–10 show. It may surmount the nucleus as a crescentic body as in figure 5, or almost completely surround it as in figure 6. Figures 5 and 6 were drawn from material fixed in Flemming's weak solution, sectioned, and stained by Heidenhain's iron-haematoxylin method, while figures 7–10 represent zoospores fixed *in toto* with osmic acid and stained directly in haematoxylin. The latter group did not stain as intensely as the former, nor is the nuclear cap as distinct. In figure 7 the nuclear cap is small and indistinct. These marked variations, although possibly due to the technique employed, or to variations in the relative amount of nuclear cap material, are more likely an evidence of the mobility of the material of which the cap is composed.

This is the first description of extra-nuclear caps in the Rhizidiaceae. Their appearance in *E. operculatum* is in general similar to those described by Thaxter (1896), Cotner (1930a), (1930b), Hatch (1935), Matthews (1937) and Karling (1937b) for *Blastocladia*, *Allomyces*, *Blastocladiella* and *Cladochytrium* respectively. The method of origin and the function of the nuclear cap are still matters of dispute. Debasieux (1920) found that following cleavage of *Coelomycidium Simuli* chromatic granules appeared in the segments, fused into a large body, and came to lie at the apex of the nucleus opposite the point of attachment of the cilium. He regarded this as an accessory nucleus, the significance of which is obscure, possibly mitochondrial in origin, having some function in the formation of the cilium. Hatch (1935) describes the nuclear cap as originating by the aggregation, vesiculation and fusion of chondriosomes around the nucleus during sporo- and gametogenesis, and believes it is equivalent to the so-called limosphere in the mosses. Karling (1937b), however, found no evidence of fusing chondriosomes in *C. replicatum* and believes that the cap may have arisen by the aggregation and confluence of chromatic granules or bodies in the cytoplasm. Thus far, I have observed no fused chondriosomes in any stage in the development of the thallus or during sporogenesis of *E. operculatum* by intra-vitam staining with Janus green. Furthermore, that the nuclear cap may be

conspicuously present in material killed in non-chondriosomal fixatives containing acetic acid and other lipoid solvents, militates against the view that it is wholly chondriosomal in origin. Thaxter and Cotner describe what is now known in *Blastocladia* as the extra-nuclear cap as lying within the nucleus, and regards it as a nutritive element, while in *C. replicatum* Karling suggests that it may be utilized as a reserve food in the germination of the zoospore. In *E. operculatum*, as will be shown more in detail later, a mass of material similar in staining reaction to that which composes the nuclear cap may be found in the germ tube and the incipient sporangia at some distance from the nucleus, which suggests that it may have been only partially utilized, and the remainder migrated into the germ tube with the nucleus during germination.

In addition to the nucleus and nuclear cap, in fixed and stained zoospores a fine cytoplasmic strand may often be seen connecting the point of attachment of the cilium with the nucleus, as in figure 6. The nuclear cap in this figure almost surrounds the nucleus, partly obscuring the exact point of contact of this strand. This cytoplasmic strand never appears as sharply defined as those figured in *Allomyces*, *Blastocladiella*, *Leptolegnia caudata*, by Hatch (1935), Matthews (1937), Cotner (1930a), and Mathews (1932). Although I have never found it to be double as in the latter genera, it is apparently of the same nature and function as the rhizoplast. At the point of insertion of the cilium on the spore is a minute dark staining region or granule, which is very similar to, but not as sharply defined, as the basal granule or blepharoplast described by Mathews (1932), Curtis (1921), Kusano (1930), and others.

The formation of the cilium was studied only in living material from observations of zoospores released prematurely from sporangia by mechanical means. Ordinarily, the cilium is visible only after the zoospores have been discharged from the zoosporangium and have begun to pull apart from the globular mass of spores. The cilium may, however, have formed earlier, for immature swarmspores forced out of the immature sporangium by mechanical pressure possess structures suggestive of developing cilia. Figures 11 and 12 show what I believe to be developmental stages which were arrested by injury. At the end of the cilium a small vesicle or loop is observed which may be the material which gradually spins out to form the cilium. Similar vesicles have been observed in *Chytridium zygnematis* by Rosen (1887) and Fischer (1892) which they believed to be loops formed by the tips of the cilia, by Kusano (1912) in *Olipidium* and regarded by him as nodules, and by Curtis (1921) and von Minden (1923) in *Synchytrium endobioticum* and *Macrochytrium*

botrydioides. All of the descriptions, however, relate to the disintegration of the cilia rather than to their development.

Biciliate zoospores are rare in *E. operculatum* and seem to be the result of unequal or incomplete cleavage rather than fusion of two uniciliate ones. In figure 13 is shown a large biciliate spore with two highly refringent globules which eventually fuse into a single large globule, as is illustrated in figure 14. Multiciliate zoospores of this type are fairly common in the Chytridiales and have been reported by Lagerheim (1888), Kusano (1912), Ojerholm (1934), Karling (1936b) and others.

Germination of the Zoospore

Germination stages of the zoospore have been studied from living material within *Cladophora* filaments, and in fixed and stained preparations made by sowing zoospores on slides smeared with a film of potato dextrose agar. The zoospores come to rest and the cilia disappear immediately before germination. In one case observed, the cilium disappeared during an amoeboid stage; whether this is the usual behavior of the zoospores cannot be stated definitely, since the process is exceedingly rapid. It is questionable whether the cilium is retracted or whether it is dropped, as Kusano (1930) has described for *Synchytrium fulgens*, and Curtis (1921) claims for *Synchytrium endobioticum*. The zoospore rounds up at once after the cilium is lost, as illustrated in figure 15, and within a few minutes produces a small papilla on the side, as shown in figure 16, which increases in length as shown in figures 17 and 18. The protoplasm changes from the hyaline, semi-transparent appearance of the active

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Explanation of Plate 1

Figs. 1–3. Zoospores, from living material, showing the various positions assumed by the highly refractive globule. Figures 1 and 2 show a structure in the center which may be the nucleus and nuclear cap (×2719).

Fig. 4. Unusually large zoospore from germinated resting spore in amoeboid phase, illustrating the plastic consistency of the highly refractive globule (×2719).

Fig. 5. Zoospore showing vacuole at anterior end, large extranuclear cap and nucleus. From material fixed in Flemming's weak and stained haematoxylin (×3495).

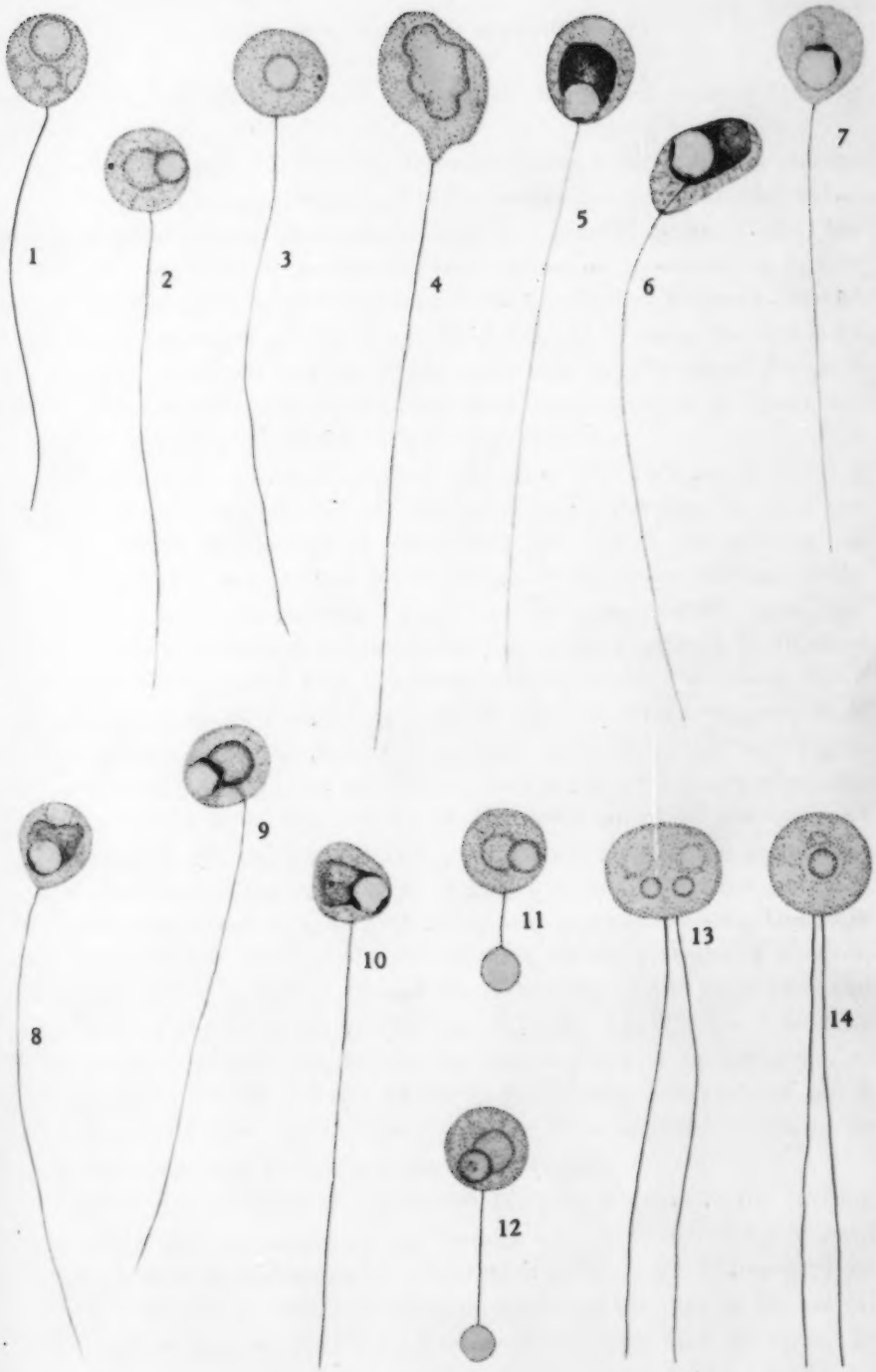
Fig. 6. Zoospore with vacuole in nuclear cap, large nucleus with nucleolus and cytoplasmic connection between nucleus and cilium. From material prepared as in figure 5 (×3495).

Figs. 7–10. Zoospores from material fixed in osmic fumes and stained with iron haematoxylin showing the nucleus and various amounts of extranuclear material surrounding the nucleus (×3495).

Figs. 11, 12. Immature swarmspores showing possible cilium development (×2719).

Fig. 13. Biciliate zoospores with two highly refractive globules (×2719).

Fig. 14. Same as figure 13, a little later, after fusion of highly refractive globules (×2719).



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zoospore to a slightly greyish color with the still-distinct refractive globule.

The nucleus and nuclear cap likewise undergo some optical change and are not so sharply defined as in the active zoospore. In the initial stages of germination, observed in fixed and stained preparations, the nucleus and nuclear cap remain in the zoospore as illustrated in figures 19 and 20. The germ tube develops as a fine filament, as in figure 21, and often attains a length of 43μ before branching or forming the rudiment of the zoosporangium. The tip of the germ tube usually forms the main axis of the rhizoidal system and branching may occur, as in figure 22, before the appearance of the incipient sporangium.

The spores often form more than one germ tube on agar as Karling (1937a) has already shown, but the sporangium develops in only one of them. Which of these germ tubes shall give rise to the sporangium seems to be determined by the final position of the spore nucleus. If the zoospore is binucleate, however, and forms two or more germ tubes, two sporangia may be formed, provided that the nuclei migrate into different tubes, but I have never seen this occur. Occasionally the young thalli have the appearance shown in figures 32 and 46, which suggests that branching may have occurred at the side of the rudimentary sporangia. But, as Karling (1931) has pointed out in the case of *Entophlyctis*, this appearance may have come about by the development of the incipient sporangium at the juncture of two or more branches of the germ tube. As the rudiment of the sporangium begins to enlarge, the branches are carried farther apart so as to give the appearance noted above. Branches may actually grow out from the rudiments in the early stages of development, as is shown in figures 32 and 46. Branching of the germ tube and the genesis of the rhizoidal system may take place before the formation of the swelling which is to become the sporangium, as in figure 22, or after, as in figures 24 and 25. As far as my present observations go, it seems to occur before, as has been shown for *Diplophlyctis*, *Entophlyctis* and *Nephrochytrium* (Karling 1930, 1931, 1938a).

The nucleus and nuclear cap do not begin to migrate to the swelling immediately upon germination, but usually remain in the zoospore until the germ tube is well developed, as illustrated in figure 27. It has not been possible to establish a definite relationship between the time of the arrival of the nucleus and the local enlargement of the germ tube. In figure 26 the nucleus is shown in a long germ tube and in figure 28 two nuclei are shown in the branched germ tube, but as yet no swelling has occurred.

Figures 29 to 32 show conspicuous swellings, but it is impossible to tell whether these were formed before or after the arrival of the nuclei. In figure 33, although a definite swelling is already apparent in the germ tube, the nucleus and nuclear cap are still within the zoospore. This would indicate that the initial stages of swelling are not dependent upon the presence of the nucleus in that region. In figure 28 the lower nucleus in the germ tube may have a nuclear cap or some extra-nuclear material on one side, and between the second nucleus and the old zoospore is also a large amount of heavily staining material. Whether or not this is extra-nuclear cap material which has been carried down with the nuclei is not certain, but it is quite chromatic and stains intensely like the nucleole. Binucleate thalli like those in figures 28 and 32 occur rarely

Explanation of Plate 2

Fig. 15. An unusually large zoospore from resting spore sporangium rounded up ready for germination (×1040).

Figs. 16–18. Stages in the development of the germ tube (×1040).

Fig. 19. Early stage in germination, from fixed and stained material showing nucleus and nuclear cap (×1040).

Fig. 20. Early stage similar to figure 19 but greatly magnified (×2330).

Fig. 21. Stages in germination, from living material showing the long filamentous germ tube (×1040).

Fig. 22. Branching of germ tube to form the initial rhizoidal system (×1040).

Fig. 23. Same as figure 22 a little later showing the initial swelling in the germ tube (×1040).

Fig. 24. A slightly later stage in which the contents of the zoospore have migrated into the sporangium (×1040).

Fig. 25. Initial swelling in unbranched germ tube (×1040).

Fig. 26. Early germination stage, from fixed and stained material showing the migration of the nucleus. (×1040).

Fig. 27. Long germ tube with nucleus remaining in the zoospore (×1040).

Fig. 28. Germination stage showing two nuclei migrating into the germ tube (×1040).

Fig. 29. Early germination stage, from fixed and stained material showing nucleus in incipient sporangium (×1040).

Fig. 30. Type of branching of germ tube which is frequently found when zoospores germinate on agar (×1040).

Fig. 31. Normal type of rudimentary thallus, with the nucleus in the swelling and a large amount of extranuclear chromatic material in the old zoospore case. (×1040).

Fig. 32. Binucleate incipient sporangium (×1040).

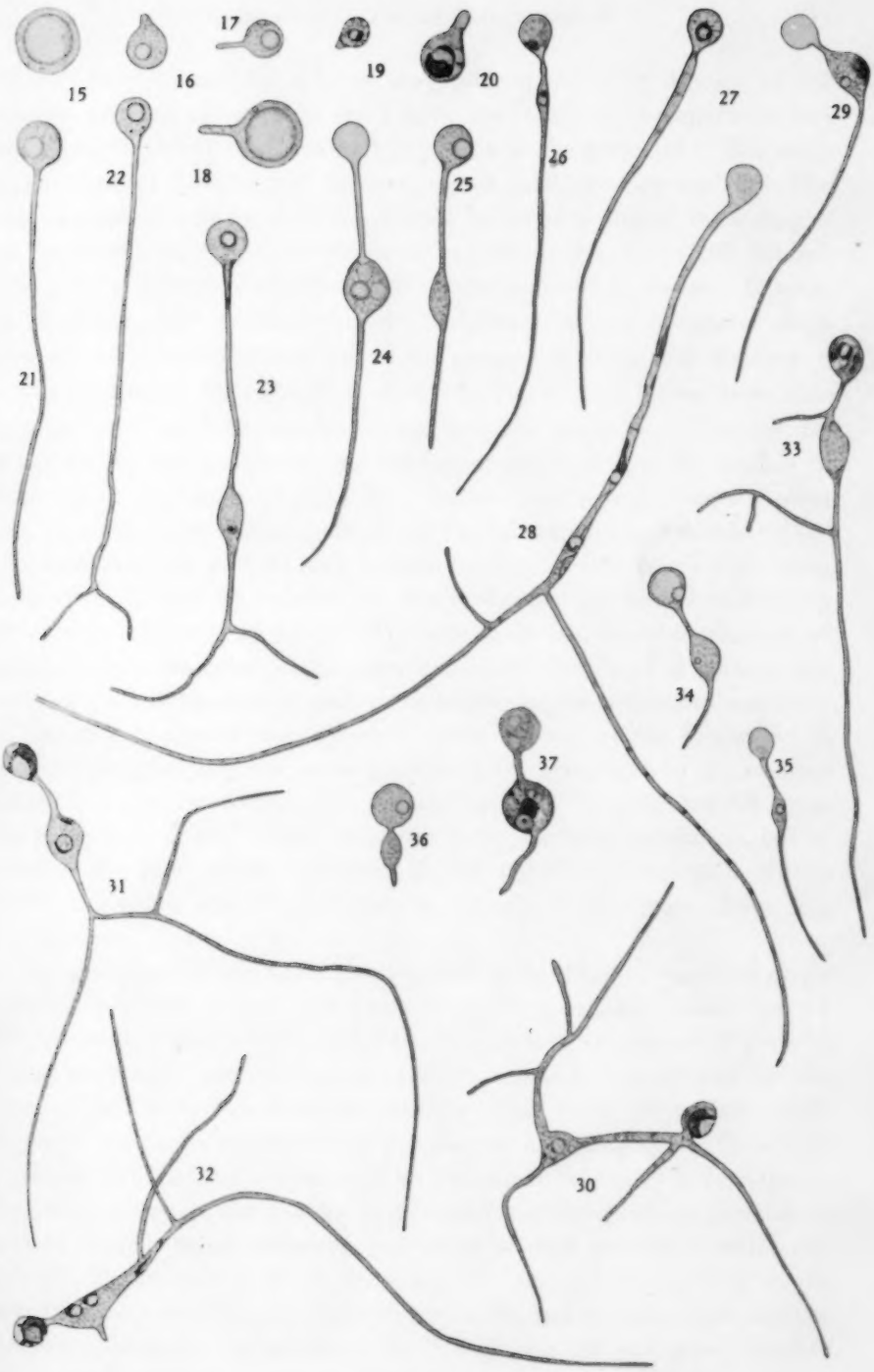
Fig. 33. Germination stage from living material showing gradual vacuolization of the zoospore (×1040).

Fig. 34. An early germination stage, from fixed and stained material showing the enucleate, primary swelling (×1040).

Fig. 35. An early germination stage showing the nucleus in the primary swelling. The relative sizes of the nucleus and germ tube indicate that nucleus probably elongates during its migration through the germ tube. (×1040).

Fig. 36. Sporangium developing adjacent to the zoospore without the formation of a long germ tube (×1040).

Fig. 37. Germination stage, from material grown in *Nitella*, and fixed and stained showing nucleus and extranuclear chromatic material in the swelling (×2330).



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and may have arisen from a large binucleate spore or by division of the zoospore nucleus, although so far I have not found the zoospore nucleus dividing in the spore itself or during transit in the germ tube. The more normal type of development is that shown in figures 29 and 31. The large amount of heavily stained material in the zoospore of these figures and figure 30 suggests that perhaps the nuclear cap had been left behind, since none is visible around the nuclei in the incipient sporangia. It may, nevertheless, relate to the refractive substance of the zoospore which has not been completely dissolved in the process of fixing and staining.

Vacuolation of the cytoplasm occurs as the nucleus moves down into the germ tube, until the zoospore case becomes empty, as in figures 24, 28 and 29. In living material the refringent material usually remains in the zoospore until most of the other contents have moved down the germ tube, as in figure 34. Small amounts of the substance are observed in the germ tube, and the globule may become broken up into smaller globules, as Karling (1930) has shown in *Diplophlyctis*. As has been already noted with reference to figure 28, there is a considerable amount of densely stained material in the germ tube, and strands of it between the two nuclei, all of which is probably the residue of the refringent material.

The appearance of the nucleus in figure 26 and of the two nuclei in figure 28 suggests that the nuclei pass down the germ tube to the swelling without any apparent change in size or shape. In these figures the germ tube is quite large and readily accommodates the spherical nuclei, but in instances like those shown in figures 31, 35 and 37 the nucleus doubtless became elongated and very slender in transit, as has been shown for *C. replicatum*.

The germination and early development of the thallus described above appears to be the normal and usual type. In a manner unlike that of *Rhizophidium*, *Tylochytrium* and *Phlyctochytrium*, the center of growth, development and organization of *Endochytrium* is transferred by the migration of the nucleus from the zoospore itself to an intercalary swelling which eventually becomes a sporangium or a resting spore. This type of germination and the provision of an intramatrical center is perhaps an adaptation to place the thallus in a more favorable position relative to its food supply. When zoospores are liberated and germinate within the host cell, the distance between the zoospore case and the swelling in the germ tube may be short, as shown in figure 36, and in some cases lacking altogether, whereby the zoospore itself may enlarge and grow directly into a sporangium or a resting spore. I have not actually observed the

developmental stages of such thalli, but the presence of a number of resting spores closely packed together in an old sporangium in figure 100 suggests that they have been formed directly from the zoospores. In such exceptional cases we would thus have the same type of development as in *Rhizophidium*, *Tylochytrium* and *Phlyctochytrium* where the enlarged zoospore becomes the center of growth, development and organization. Certain genera of chytrids doubtless have a predominant type of development which, however, may vary under special conditions and approach that of another type. In *Rhizophlyctis*, for example, the sporangium is reported to have grown directly from the zoospore occasionally, and in *R. Liguicola* (Lindau) Minden it may form as an outgrowth of the zoospore.

Development of the Sporangium

After germination of the zoospore and the establishment of the young thallus, the swelling begins to enlarge into the incipient zoosporangium. This enlargement seems to keep pace with the growth, branching and extension of the rhizoidal system. I have not studied their relative growth rates, but evidently the growth of the sporangium is more rapid after the rhizoidal system has become well established. Following its arrival in the incipient sporangium, the nucleus usually enlarges to a diameter of 3.5μ to 5μ before division, without showing any marked changes in structure. The nucleus before enlargement is spherical and almost empty except for a crescentic nucleolus surrounded occasionally by a well defined nuclear cap as is shown in figure 46. Figure 47 shows an intermediate stage in nuclear enlargement, and figure 48 a later stage in which the primary nucleus has almost doubled its size. With the increase in size of the sporangium and nucleus, the cytoplasm appears to be more vacuolate probably

Explanation of Plate 3

Fig. 38. Initial stage in the development of the sporangium as observed in living material (×1560).

Fig. 39. Enlargement of the developing sporangium with large vacuole and highly refractive globules (×1560).

Fig. 40. A later stage showing an increase in the number of globules (×1560).

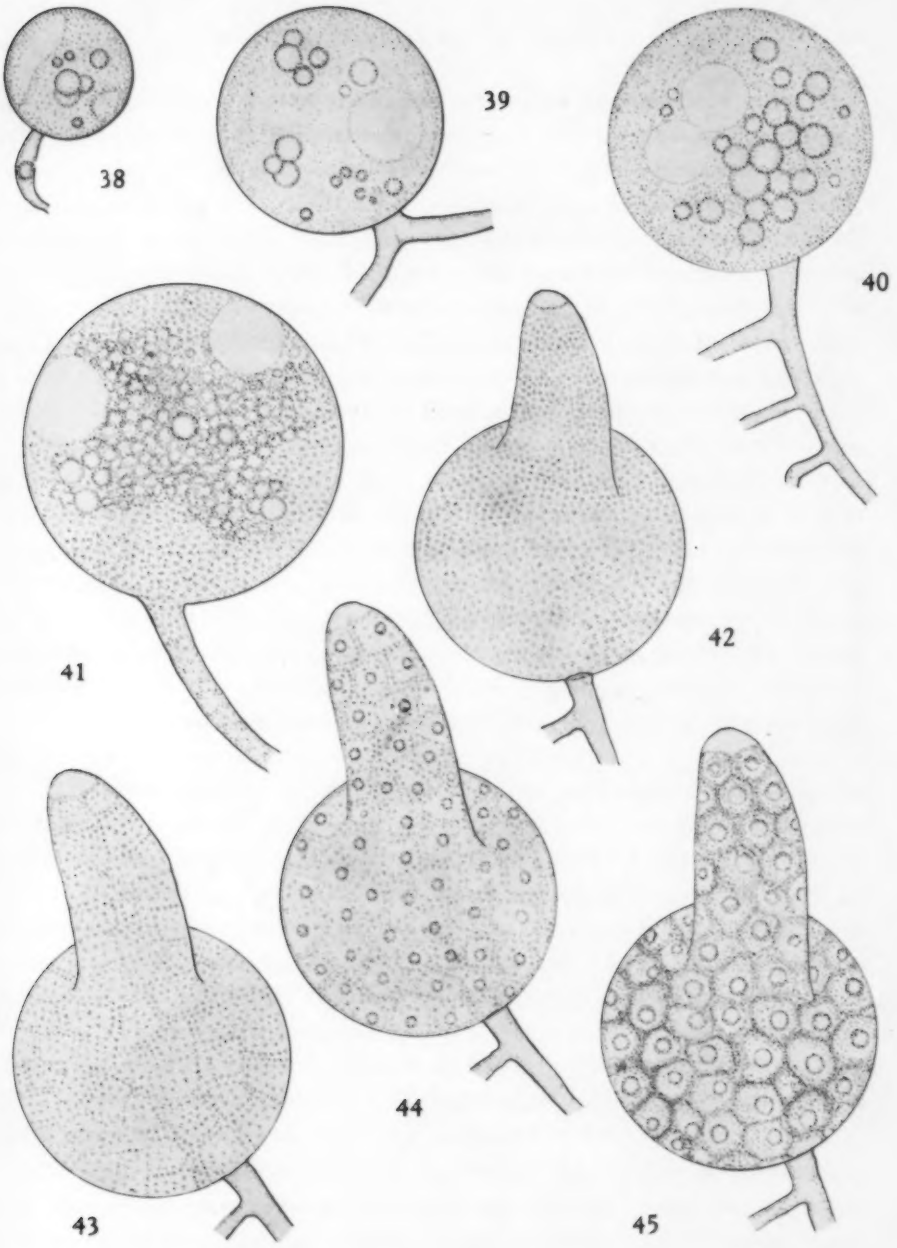
Fig. 41. Second stage in the development of the sporangium showing the gradual dispersion of the highly refractive globules (×1560).

Fig. 42. Third stage of sporangial development showing the refractive material in the highly dispersed, granular stage (×1560).

Fig. 43. Beginning of third or maturation stage of sporangial development. Faint lines suggesting cleavage have appeared in the finely granular protoplasm (×1560).

Fig. 44. Later stage showing formation of highly refractive globules of the zoospore initials (×1560).

Fig. 45. Mature sporangium containing zoospores ready to be discharged (×1560).



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because of the dissolution of the highly refractive globules during fixation. While enlargement of the sporangium proceeds, as observed in living material, there is an increase in the amount of highly refractive material as shown in figures 38, 39, 40. On the basis of size, figure 38 corresponds to figure 50 or 51 of the fixed and stained thalli, while figures 39 and 40 may be comparable to figure 52. Except for comparative size, I have not found any criterion by which to connect stages in the living material with similar stages in the fixed and stained preparations. I shall, therefore, give a brief description of sporangial development in living material and supplement it with my observations on fixed and stained preparations.

The development of the evanescent sporangium may, for convenience of description, be divided into three stages according to the character of the protoplasm, particularly of the highly refractive substance. The first stage may be considered to be the local swelling of the germ tube and the arrival of the nucleus, as shown in figures 29 and 31, a stage characterized by an increase in the amount of highly refractive substance coincidental with the enlargement of the sporangium as shown in figures 38 to 40. The highly refractive material in the form of globules tends to collect in the hyaline protoplasm near the center of the sporangium. One or more large vacuoles may be present, as shown in figure 40.

The second stage in the development of the sporangium relates to the gradual dispersion of the highly refractive globules. As the sporangium enlarges, the globules in their central arrangement become irregular in shape, as shown in figure 41, and break up imparting to the hyaline protoplasm of the maturing sporangium an even and finely granular appearance, as shown in figure 42. The protoplasm at this time is greyish-white as Karling (1938d) has observed in a similar stage of *Rhizophidium laterale*. The optical appearance of the process of dispersion seems to be similar to that described by Couch (1935) in *Phlyctidium anatropum*. He describes it as a digestion of the large globules and questions his own description of these bodies as being composed of fat.

The third stage, illustrated in figures 43–45, relates to maturation and zoosporogenesis. The minute granules formed in the second stage appear to coalesce, forming slightly larger granules. These arrange themselves, as in figure 43, chiefly along the borders of segments which may represent the zoospore initials.

A similar condition has been shown in *Rhizidiomyces apophysatus* and *Diplophlyctis intestina* by Zopf (1884) and in *Nephrochytrium* and *Endochytrium digitatum* as observed by Karling (1938a, 1938b). Figure

44 shows zoospore globules. Ultimately, as shown in figure 45, all the refractive substance becomes localized in the characteristic globules of the zoospore. This process of coalescence has been described in the development of the zoospores of *Polyphagus Euglenae* (Wager 1913), *Rhizophidium globosum* (Couch 1932) and *R. Lagenaria* (Sparrow 1936). The zoospores in figure 45 are about to be discharged from the zoosporangium and, probably because of their imbibition of water, they are pressed together firmly in somewhat polygonal shapes.

At the tip of the exit tube is a clear region that emits a hyaline viscid fluid at the time of the discharge of the zoospores. In the sporangium from which figures 42–45 were drawn, the operculum did not become noticeably thickened, but it was clearly evident following the discharge of the zoospores.

The early developmental stages as seen in fixed and stained material are shown in figures 46 to 52. These seem to correspond to the first stage described in the living material. In figure 46 the nucleus has arrived in the swelling, and in figures 46–47 it is increasing in size until in figure 48 it has become three times the size of the zoospore nucleus. The cytoplasm in some cases, as in figure 47 and the binucleate sporangia of 49 and 50, may be vacuolated. The four and sixteen nucleate stage is shown in figures 51 and 52. Since the cytoplasm in this stage does not contain the large vacuoles present in figures 39–41 of the living material, it is assumed that this is a later stage than that drawn in figures 39–41.

It is noticeable that no nuclei have been present in the rhizoids. Figure 50 is merely an apparent exception. In the basal part of this rhizoid there is a heavily stained body which might resemble a third nucleus, but since division is simultaneous within the sporangium, it would be expected that either two or four nuclei would be present instead of three. The only explanation would be that division in one of the original two nuclei had been delayed. It is my belief that this body is chromatic

Explanation of Plate 4

Fig. 46. Early stage in the development of the thallus, soon after the arrival of the nucleus in the incipient sporangium, from fixed and stained material (×920).

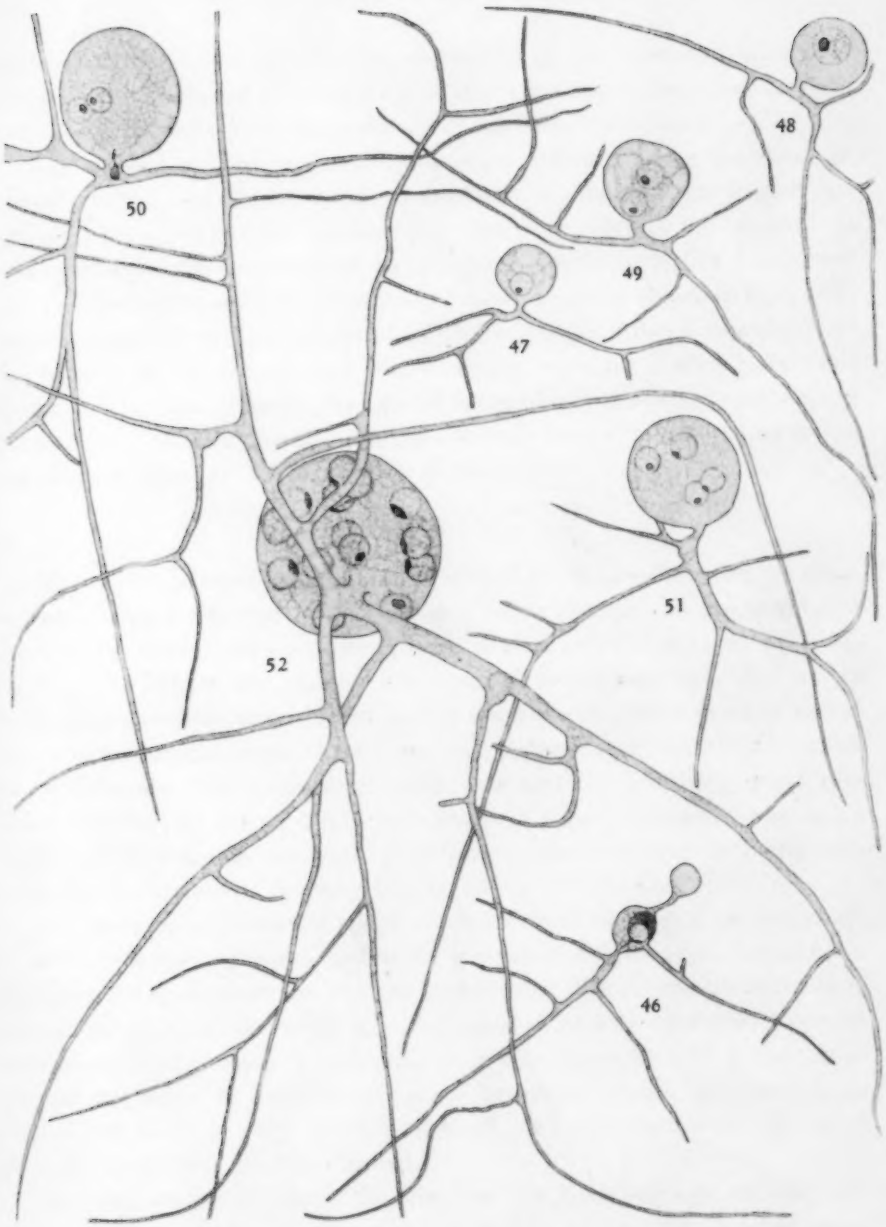
Figs. 47, 48. Later uninucleate stages in the development of the thallus with the nucleus increasing in size (×920).

Fig. 49. Binucleate stage of the developing thallus (×920).

Fig. 50. Binucleate stage in the development of the thallus showing possible extranuclear material in the base of the rhizoid (×940).

Fig. 51. Four-nucleate stage with enucleate rhizoid (×920).

Fig. 52. Sixteen-nucleate stage. The rhizoid is continuous with the sporangium and no nuclei have yet appeared in the rhizoid (×920).



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material which has remained in the rhizoid, since the examination of over one hundred additional whole mounts of thalli in various stages of development has failed to reveal the presence of nuclei in the rhizoids.

The rhizoid is continuous with the sporangium during the developmental stages, and its protoplasm contains highly refractive globules, but as the sporangium reaches the granular stage, the rhizoid is separated from the sporangium by a septum and is optically homogeneous. Furthermore, in fixed and stained preparations as shown in figure 71, there is some cytological evidence of protoplasmic flowing especially at the basal part of the rhizoid where deeply staining bodies have been drawn out to form strands. In view of these observations it seems logical to conclude that the protoplasm has flowed from the rhizoid into the sporangium prior to the formation of the septum.

Nuclear Division

Nuclear division in the evanescent sporangia is simultaneous, as illustrated in figure 68, and mitotic. The division spindles in figure 68 are oriented in several planes so that both profile and polar views of equatorial plate stages are visible. The spindle is intranuclear, and at its poles characteristic cone-shaped bodies are present. Since distinct astral rays are frequently oriented on these bodies, the latter doubtless function as centrosomes. The vacuolated cytoplasm and the relatively great distance between the nuclei would indicate that this sporangium was in an intermediate stage in development which is characterized in living material by the presence of many highly refractive globules.

In figure 53 is shown a nucleus which I believe to be in a resting stage or in a very early prophase. Within the nucleus is a large, lens-shaped nucleolus lying against the nuclear membrane. The chromatin reticulum, consisting of irregular strands, is faintly stained and appears in a more continuous background. The nucleus shown in figure 54 with a flat, ring-shaped nucleolus is possibly an early prophase stage. The chromatin bodies are larger, more sharply defined, and are connected by faint strands which may be linin threads.

Spireme stages in which the nucleole lies flattened against the side of the nuclear membrane are shown in figures 55 and 56. A later stage is shown in figure 57. The thick, irregular and elongate strands are clearly defined and look like the early stages of chromosome formation. These become more distinct and stand out as short, elongate rods before there is any indication of the achromatic spindle, as is shown in figures

58 and 59. The ring-shaped nucleolus is prominent in figures 58 and 59, and in figure 60 in an optical view is shown to be vacuolated. The ring-shaped nucleolus as found in *E. operculatum* is very similar to that described by Karling (1937b) for *Cladochytrium replicatum*. In the metaphase the nucleolus is reduced to a round, flattened body which lies at the nuclear equator and against the membrane, as shown in figure 63. It appears to differ in size and shape in various nuclei, becoming quite indistinct in figure 65 and in the anaphase shown in figure 66, but quite prominent in the late anaphase shown in figure 67.

As has been noted above, densely staining, cone-shaped bodies may be found at the poles of the nucleus and the division figure as in figure 56a. These structures may be initial stages in the formation of the central bodies which are prominent at the poles of the spindles in figures 61 and 62 and particularly in figures 63–65. The central bodies are not as sharply defined as those shown by Harper (1897) and (1905) in *Erysiphe* and *Phyllactinia* and do not have such bushy and dense astral radiations. In figure 61 the astral rays are short, while in figure 62 they are long. The central bodies shown in figure 64 resemble those of *Polyphagus Euglenae* (Wager 1913). Centrosome-like bodies have been reported by Hovasse (1936) at the poles of the intra-nuclear spindle of

Explanation of Plate 5

Fig. 53. Resting nucleus with lens-shaped nucleole and faintly staining chromatin reticulum (×4167).

Fig. 54. Prophase stage with chromatin reticulum showing the ring-shaped nucleolus (×4167).

Figs. 55, 56. Spireme stages.

Fig. 57. Late prophase stage (×4167).

Figs. 58, 59. Late prophase stages showing chromosomes (×4167).

Fig. 60. Face view of the ring-shaped nucleolus in prophase stage (×4167).

Fig. 61. Prophase stage with central bodies and short astral rays (×4167).

Fig. 62. Prophase stage with central bodies and extensive astral rays (×4167).

Figs. 63, 64. Metaphase stages showing central bodies at the poles of the intranuclear spindle (×4167).

Fig. 65. Metaphase stage showing poles of intranuclear spindle extended through the nuclear membrane (×4167).

Fig. 66. Anaphase stage (×4167).

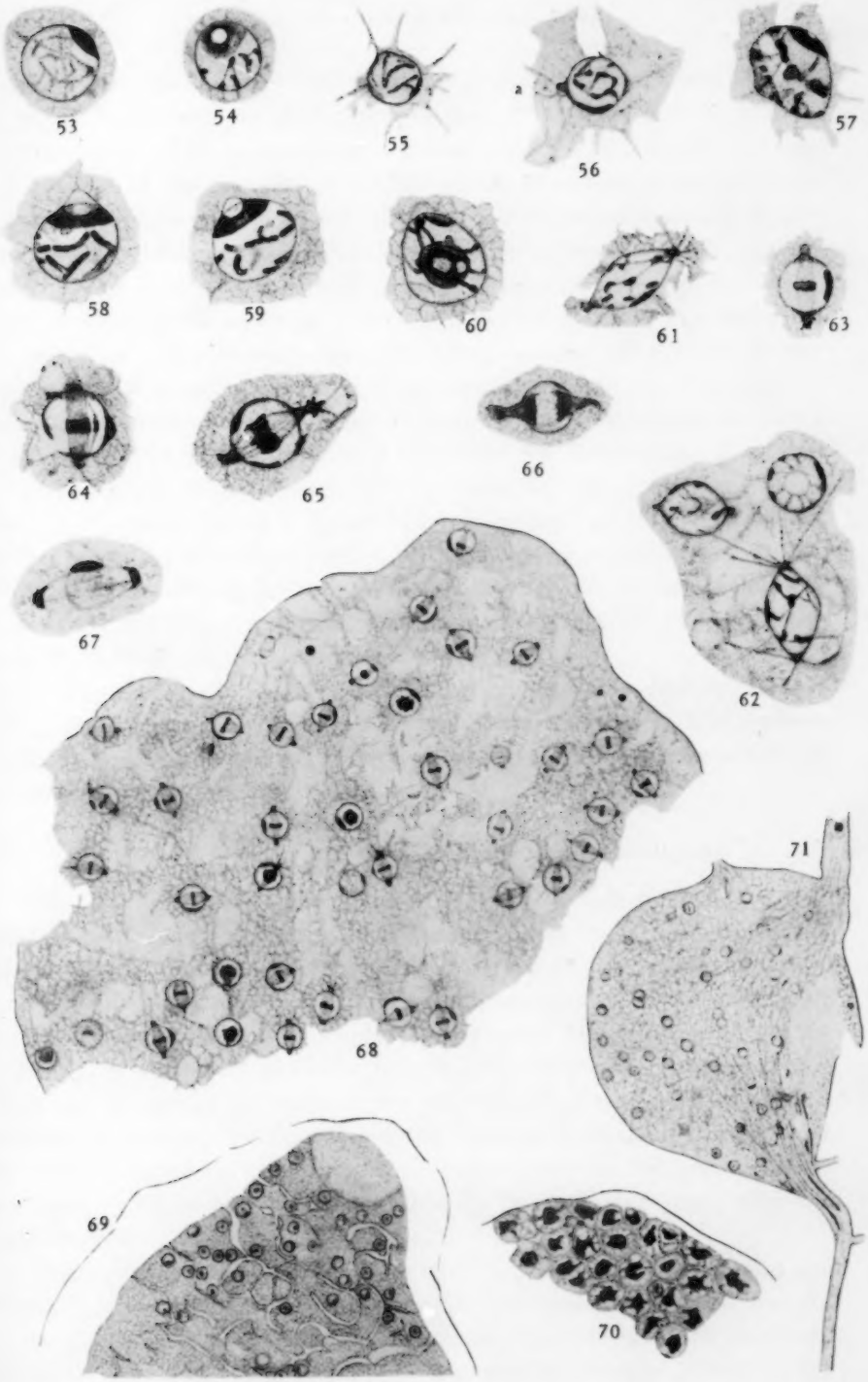
Fig. 67. Late anaphase, region of old nucleus still discernible (×4167).

Fig. 68. Section of evanescent zoosporangium showing simultaneous nuclear division (×1300).

Fig. 69. Progressive cleavage of protoplasm of evanescent zoosporangium (×1300).

Fig. 70. A portion of a section through a zoosporangium showing polygonal zoospore initials (×1300).

Fig. 71. Section through developing evanescent sporangium showing lines of flow of protoplasm from the rhizoids (×1300).



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Rhizophidium Beauchampi but these are rather indistinct and do not appear to resemble those of *E. operculatum.*

The spindle of *E. operculatum* is intra-nuclear, a characteristic common to most of the chytrids except *Olipidium Brassicae* (Nemec 1912), *O. radicale* (Schwartz and Cook 1928), *Cystochytrium radicale* (Cook 1932) and the resting spores of *Olipidium Vicae* (Kusano 1912), species which belong in the Myxochytridineae. The chromosomes in the equatorial plate stage shown in figures 63 and 65 are so close together that the individual chromosomes are not distinguishable. The poles of the spindle appear to extend through the nuclear membrane but this appearance may be caused by the large central bodies at these points. This spindle structure is similar to that described for *Polyphagus Euglenae* (Wager 1913) in which the astral rays extend through the nuclear membrane. The spindle shown in figure 64 has a reduced central body similar to that shown by Karling (1937b) in *Cladochytrium replicatum.* In the anaphases, the poles of the spindle extend through the nuclear membrane as in figure 66. The blunt, irregular apex of the spindle suggests that restricted astral rays may be present.

A late anaphase stage is shown in figure 67 with a heavily stained chromosome group now lying at the poles of the spindle. The nuclear cavity is faintly outlined in the cytoplasm, possibly by the remnants of the nuclear membrane.

Zoosporogenesis in the primary evanescent sporangium

The cleavage of the sporangium of *E. operculatum* to form the uniciliate zoospores is by progressive furrowing. Since the process of delimitation of the zoospores in this species is essentially similar to that described for many algae and fungi with sporangia, it will be dealt with briefly. Cleavage of the protoplast apparently begins soon after the large highly refractive granules have become dispersed throughout the entire cell as minute granules in the way described earlier. The hyaline protoplasm makes identification of the cleavage furrows uncertain and the evidence based entirely upon the reorganization of the granules does not seem to me to be sufficient to identify the granular stage with the beginning of cytokinesis.

Cleavage furrows, as they appear in fixed and stained material are shown in figure 69. The furrows proceed centripetally from the margin of the protoplast and in various directions from clefts which originate within the center of the protoplast as described by Harper (1899) in the

formation of the protospores of *Pilobolus Crystallinis*, *Synchytrium decipiens* and the spores of *Sporodinia grandis*. They are likewise similar to those of *Rhizopus nigricans* and *Phycomyces nitens* Swingle (1903). Progressive cleavage as found in *E. operculatum* agrees with most observations on the Phycomycetes and since these have been summarized frequently by Harper (1899), Swingle (1903), Schwartz (1922), Bold (1933) and Karling (1937b) they need not be discussed here.

The zoospore initials soon after their formation may assume a polygonal shape as shown in figure 70 which may be comparable to the mature stage of the living material shown in figure 45. This shape is due to pressure resulting from the intake of water following cleavage such as Harper (1899) has found to occur in *Synchytrium decipiens* and *Sporodinia grandis*. Schwartz (1922) has observed the zoospores of *Olpidiopsis Saprolegniae* to become swollen following their delimitation, a phenom-

Explanation of Plate 6

Figs. 72–80. Stages in the development of a smooth-walled resting spore, from living material, showing at intervals the fusion of the highly refractive globules (×975).

Fig. 81. Mature smooth-walled resting spore showing large central refringent globule (×975).

Fig. 82. Mature resting spore showing the empty zoospore case with germ tube and unbranched rhizoid (×975).

Fig. 83. Early stage in resting spore formation, from material fixed and stained *in toto* (×975).

Figs. 84–86. Developmental stages of resting spore, from fixed and stained material, showing increased vacuolation of protoplasm (×975).

Fig. 87. Median optical section of mature resting spore, showing nucleus in peripheral layer of protoplasm (×975).

Fig. 88. Resting spore in advanced stage of development showing a binucleate condition (×975).

Fig. 89. Small mature resting spore showing chromatic granules in the cytoplasm (×957).

Fig. 90. Surface view of protoplasmic layer of mature resting spore, from fixed and stained material (×975).

Fig. 91. Median optical section of living mature resting spore which has developed within an evanescent sporangium and showing protoplasmic layer surrounding the central globule (×975).

Fig. 92. Surface view of protoplasmic layer of living resting spore (×975).

Fig. 93. Germinated resting spore showing the three layers of the wall and empty sporangium (×975).

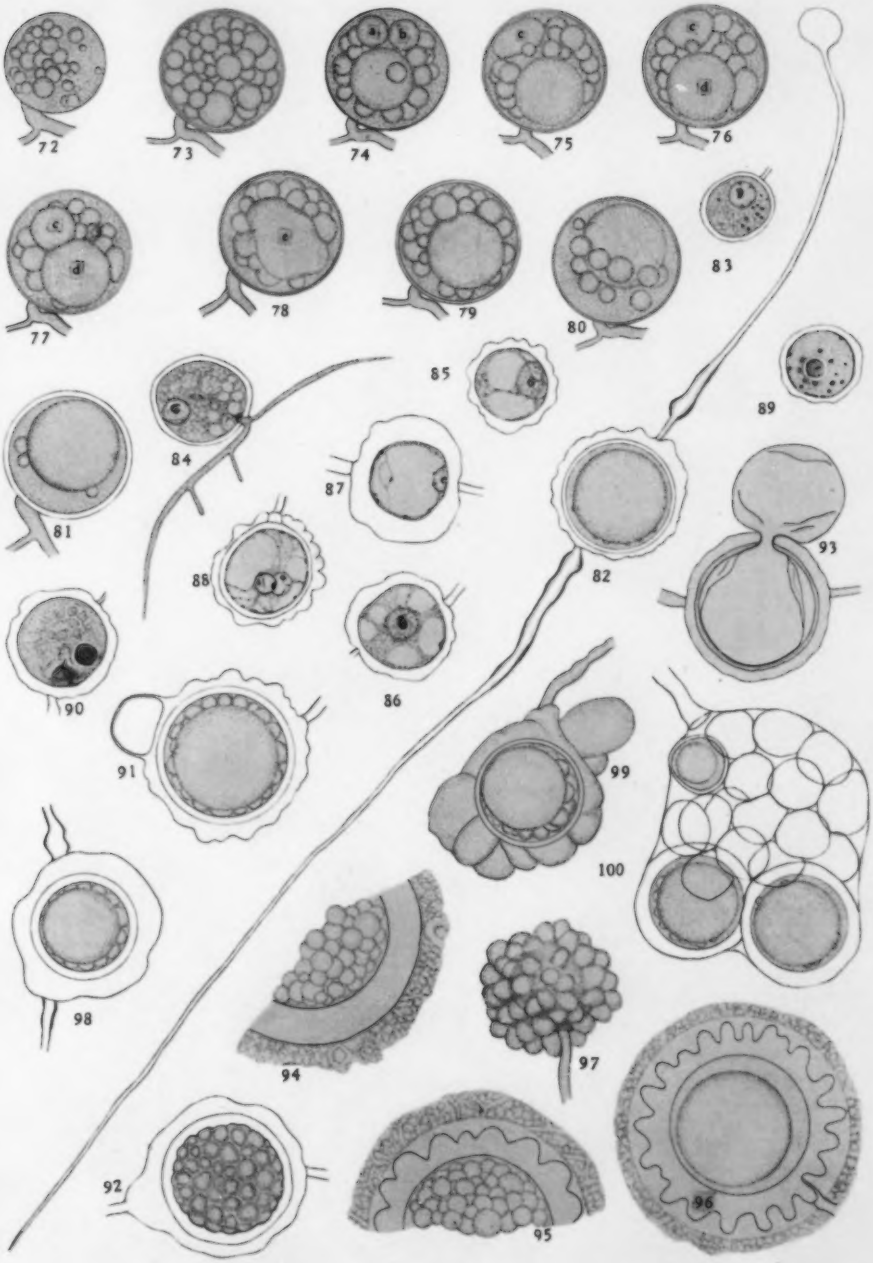
Figs. 94–96. Stages in the formation of a rough wall of a resting spore showing the dead host protoplasm and the shrinkage of the initial wall from it (×975).

Fig. 97. Surface view of rough-walled resting spore (×975).

Fig. 98. Median optical section of thick-walled resting spore, from living material (×975).

Fig. 99. Median optical section of resting spore showing heavily lobed wall (×975).

Fig. 100. Resting spores and degenerated zoospores within an evanescent sporangium (×975).



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enon which appears to occur frequently in the maturation of Oomycetes and Zygomycetes.

The nuclear cap material is present in the zoospores of the polygonal stage shown in figure 70 but it was apparently not present in the initial cleavage stage of figure 69. This would indicate that nuclear cap formation in *E. operculatum* takes place following the initiation of cleavage furrows and at about the same stage in development as the deeply stainable material as observed in the zoospores of *Polyphagus Euglenae* (Wager 1913). Similarly the nuclear cap of *Allomyces arbuscula* (Hatch 1935) and *Cladochytrium replicatum* (Karling 1937b) appears to become discernible following the initial cleavage stage.

The structure and germination of the resting spore

The development of the resting spore.—The resting spores of *E. operculatum* were described by Karling (1937a) as being smooth or rough-walled, and my study confirms these observations. As is shown in figures 81, 91 and 97, the wall may be smooth and relatively thin or thick, rough and warted. The thickening of the wall may take the configuration of lobes, as in figure 99, or it may be very irregular and uneven as in figure 98. It may even envelop a considerable part of the germ tube as in figure 92.

Resting spores form abundantly in old cultures to which no new filaments of *Cladophora* or fresh water have been added for about three weeks. Under these conditions the developing evanescent sporangia decrease in size and number and resting spores appear in increasingly large numbers. These resting spores develop either *in situ* within evanescent sporangia which fail to discharge their spores, or directly from germinating zoospores.

The young thalli which give rise to resting spores are similar to those which give rise to evanescent sporangia and cannot be distinguished until the local enlargement of the germ tube has reached considerable size. The first indication of resting spore development is a gradual increase in the number of highly refractive globules without a corresponding increase in the cell size. Figures 72 and 73 illustrate a developing resting spore observed at an interval of 19 hours. Although the structure in figure 72 might conceivably be either an evanescent sporangium or a resting spore, however, in figure 73 it is positively identified as a resting spore by the abundance of highly refractive globules. The globules coalesce gradually as is illustrated in figures 74 to 80. Two adjacent globules of equal size,

figures 74 a and b, fuse to form the elongated globule of figure 75c. This globule began to round up in figure 76c and fifteen minutes later it came in contact with the large globule of figure 77d. Fusion took place ten minutes later, momentarily forming the irregular body shown in figure 78e. Within two and one-half hours the majority of the globules had fused with this larger one as shown in figures 79 and 80. In the mature resting spore, figure 81, a few small globules which had failed to coalesce are shown at the side of the large globule. A mature resting spore thallus of limited size is shown in figure 82. The old zoospore and germ tube are still present at one side of the resting spore and opposite to this is a long unbranched rhizoid. Branching of the rhizoid occurs frequently in the resting spore but not to the same extent as in the thalli which bear evanescent sporangia. It is to be noted in figure 82 that the walls of the germ tube and the rhizoid are thickened locally but thin out at the point of attachment with the resting spore. The formation of a large number of refringent globules in the development of the resting spore and their coalescence to form one or more larger ones, as has been described above, is fundamentally similar to that already reported for *Cladochytrium replicatum*, *Chytridium lagenaria*, *E. operculatum*, *E. digitatum*, *Nephrochytrium appendiculatum*, *Chytridium aggregatum* and *Rhizophidium macrosporum* (Karling 1935, 1936b, 1937a, 1938a, 1938b, and 1938c).

In fixed and stained material the refringent globules, as such, are no longer visible and the substance of which they are composed is apparently dissolved during preparation. An early stage in the development of a smooth walled resting spore is shown in figure 83 and probably represents the condition preceding the accumulation of highly refractive globules. In figures 84 to 86 are slightly later stages from material fixed in Allen and Wilson's fixative and stained *in toto* with methyl blue and acid fuchsin in lacto-phenol. Figure 84 shows an early stage with a large number of small vacuoles in the cytoplasm which may have been left by the dissolution of the refringent globules. The nucleus of this resting spore is quite large and distinct with a conspicuous nucleole. A later stage is represented in figure 85 in which the vacuoles have increased in size and the nucleus and cytoplasm have been displaced towards the periphery of the cell, possibly by the gradual fusion of the globules. Figure 86 corresponds perhaps to figure 76 of the living material and the strands of cytoplasm shown here may be those which ran between the large globules of the living material. A mature resting spore is shown in figure 87 in which the protoplasm has been displaced to the periphery of the cell and the nucleus is

somewhat flattened against the wall. The latter figure and also figures 88 to 90 were drawn from material fixed in Allen and Wilson's and stained *in toto* in Heidenhain's haematoxylin. Surrounding the nucleus and distributed through the cytoplasm are small heavily staining granules which are also shown in figures 83, 86, 88 and 89. These appear to be similar to the basophilic and chromatic granules found in the resting spores of *P. Euglenae*, *S. fulgens* and *C. replicatum* (Wager 1913, Kusano 1930, and Karling 1937b). The resting spore shown in figure 90 is, I believe, an old one and in surface view the protoplasm appears to have the same honeycomb appearance which is often clearly evident in old living spores, as in figure 92. Figure 90 shows a view of the protoplasmic layer, which, in living material shown in median optical section in figure 91, appears as a layer of smaller globules surrounding the central mass. This layer was at first believed to consist of highly refractive globules that had not coalesced with the large one, but under microchemical tests it fails to give the same fat or oil reaction as the highly refractive substance, and is not dissolved upon fixation. In fixed and stained preparations, as shown in figures 89 and 90, a fairly large body which stains densely in haematoxylin and Feulgen's is often present in the peripheral cytoplasmic layer of such old spores.

The wall of the resting spore is composed of three layers, as shown in figure 93. The outer layer or epispore is thick and stains brilliantly with orange G., while the thinner middle wall or mesospore, indicated by the stippled region, stains blue in lacto-phenol with acid fuchsin and cotton blue. A thinner membranous endospore that may be observed in resting spores which have germinated is particularly evident in figure 93, where it has been separated from the mesospore. In the early stages in the formation of the rough or warted wall, a broad, hyaline region surrounds the resting spore and has its outer margin against the host protoplasm as shown in figure 94. Whether or not this region is derived by the secretion of a mucilagenous material as Kusano (1930) has described in *Synchytrium fulgens* or by the digestion of the host protoplasm as Miss Curtis (1921) has described in *Synchytrium endobioticum* is uncertain. The subsequent stages in the development of the wall appear to be accomplished by the gradual shrinkage and transformation of the hyaline substance, first into rather broad warts, as shown in figure 95, and later to more sharply lobed processes separated from the host protoplasm as shown in figure 96. The wall substance as it matures becomes more yellowish and opaque. It is possible, however, that the wall is not formed by the infolding of the

hyaline substance but that it develops as an outward growth from the resting spore into the hyaline region. The wall of the resting spore appears to be derived from the fungus rather than from the host protoplasm, as indicated by the fact that neutral red stains the host protoplasm heavily while the wall itself shows no reaction. This test, however, is not conclusive as it is possible that, if the wall is derived from the host, changes in its composition would occur which would also change the staining reaction. The rough-walled spore appears in median optical section as in figures 96, 98 and 99, and in surface view as in figure 97. In fixed and stained preparations the hyaline unlobed stage in wall development, figure 87, may correspond to figure 94 of the living material. At this stage the wall seems to be composed of a thick layer of hyaline material bounded on the exterior by a definite membrane. Possibly by a process of shrinking or infolding, the wall assumes the configuration shown in figure 88.

The composition of the wall has not been definitely determined. It does not stain with either chloro-iodide of zinc or iodine-potassium iodide followed by sulfuric acid. In this respect it is different from that of the evanescent sporangium, which gives a weak reaction for cellulose.

The resting spore appears to be nothing more than an incipient sporangium which has encysted and developed a thick wall in the early stages of development. It is usually uninucleate, figure 87, but occasionally binucleate as in figure 88. The latter may have arisen in one of several ways, but so far I have found no evidence that the binucleate condition

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Explanation of Plate 7

Fig. 101. Unusual resting spore formation within the rhizoid of a resting spore thallus (×865).

Fig. 102. Initial stage in the germination of a resting spore, showing the dispersion of the refringent mass (×865).

Fig. 103. Later stage in resting spore germination (×865).

Fig. 104. Advanced stage in the germination, with the highly refractive globules of the zoospores becoming evident (×865).

Fig. 105. Mature zoosporangium showing the zoospore initals completely delimited (×865).

Fig. 106. Empty zoosporangium showing the operculum (×865).

Fig. 107. A resting spore bearing a sporangium at the end of a long tube (×865).

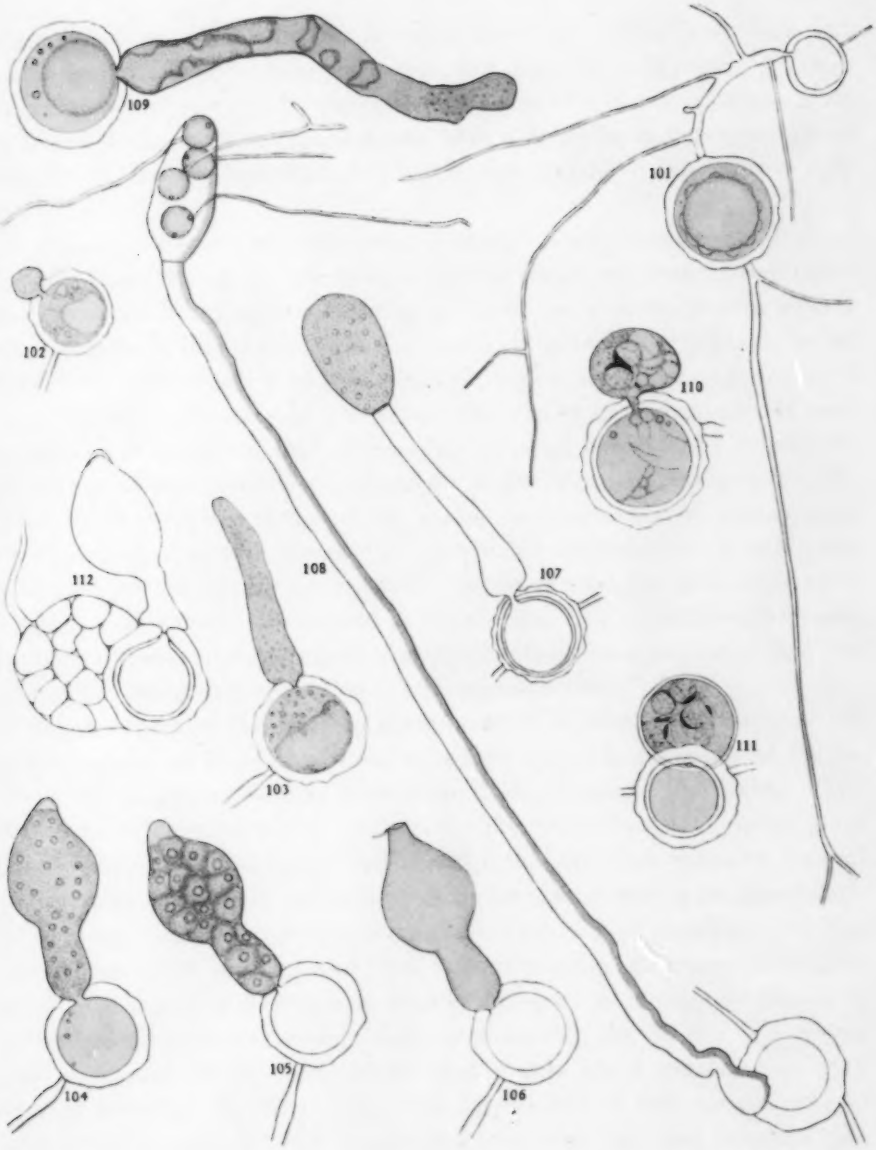
Fig. 108. Germinated resting spore in which the sporangium has formed at the end of a long tube showing zoospores germinating *in situ* (×865).

Fig. 109. Degeneration of protoplasm in tube of germinating resting spore (×865).

Fig. 110. Germinating resting spore, from fixed and stained material showing the two nuclei in the evanescent zoosporangium (×865).

Fig. 111. A four nucleate stage of the evanescent sporangium formed by the germinating resting spore.

Fig. 112. Empty zoosporangium of a resting spore extending beyond the wall of the evanescent sporangium in which the resting spore had formed (×865).



HILLEGAS: CYTOLOGY OF ENDOCHYTRIUM

is the result of sexual fusion. Biciliate zoospores which may look like motile biciliate zygotes have been seen, but these have apparently arisen as the result of incomplete cleavage. Instances of two nuclei in the germ tube and in the incipient sporangium have already been shown in figures 28 and 32, and the binucleate resting spores may have arisen from such thalli.

Figure 100 shows an interesting and significant occurrence of three resting spores within an old zoosporangium which apparently has failed to open. Such sporangia containing as many as a dozen resting spores have frequently been found in my material. It is to be particularly noted that there is no evidence of rhizoids, and the resting spores appear as if they had arisen directly by the mere enlargement of a zoospore. If such is the case, it is obvious that *E. operculatum* may occasionally exhibit the same type of monocentric development as the Myxochytridineae. On the other hand, another variation of thallus organization of rather rare occurrence is shown in figure 101 in which polycentricity is exhibited. A smooth walled resting spore has developed nearest the germ tube while a rough walled spore has formed in the rhizoid. The smooth walled spore is empty, which might suggest that the contents had migrated into the rhizoid to establish a second center of reproduction.

Germination of the Resting Spore.—In February, 1938 germinating resting spores of *E. operculatum* were first found in filaments of *Cladophora* which had previously been cleared in potassium hydroxide. The first step in germination is apparently the formation of a small germ pore in the wall of the spore, and through this aperture extrudes a small vesicle filled with finely granular protoplasm as is shown in figure 102. At the same time the large refractive globule appears to divide into smaller units, figures 102 and 103, and these in turn become finely divided to form small granules. The protoplasm within the spore is homogeneous except for the large, irregular mass which is apparently the residue of the central refringent body. The vesicle that forms upon germination may develop directly into the sporangium, figures 103 to 106, or elongate to form a tube the tip of which is delimited by a cross wall and develops into a sporangium, as is shown in figures 107 and 108. This long tube is evidently an adaptation upon the part of the fungus to discharge its zoospores to the outside of the host cell. The protoplasm in the incipient sporangium is quite uniformly granular. Not many of the stages of germination have been observed in fixed and stained preparations but from the results obtained it appears that mitosis occurs in the sporangium

and not in the resting spore. So far no uninucleate stages of the incipient sporangium have been found, but in figure 110 is shown a binucleate stage. This condition may have arisen by the germination of a binucleate resting spore, such as is shown in figure 88, or by division of the primary nucleus after its migration into the incipient zoosporangium. I am inclined to take the latter viewpoint. That further mitoses occur in the development of the sporangium rather than in the resting spore is suggested by figure 111 which shows a tetra-nucleate stage. The stages which have so far been found correspond closely with those of Wager (1913) for *P. Euglenae.* Figures 104 and 105 show successive stages in the maturation of a fully formed sporangium. The processes of maturation and zoosporogenesis here are very similar to those described for the evanescent primary sporangium. The protoplasm is uniformly granular in the late developmental stages, and then the minute refringent granules appear to coalesce gradually, figure 104, to form the large refractive globule of the zoospore, figure 105. The clear region evident beneath the operculum of the evanescent sporangium, is likewise present in, and characteristic of these sporangia. The cleavage lines in the early stages are difficult to see, but later, as in figure 105, they may be distinguished fairly well. The zoospores are discharged through an operculate opening, figure 106, flow out in a globular mass, and lie quiescent for a short time before becoming active and swimming away. They are in all respects similar to those produced by the evanescent sporangium. In exceptional cases when the operculum fails to push up, the zoospores are discharged at the edge of the operculum, as is shown in figure 112. This figure shows a germinated intra-sporangial resting spore with the new sporangium developed through the wall of the old evanescent sporangium. As has been noted above, the germinating resting spore may sometimes form a long tube, instead of a globular sporangium, which swells up at the tip, as is illustrated in figures 107 and 108. As all or most of the protoplasm has moved up into the swollen tip, it is delimited by a cross wall, in the same manner as De Bary (1884) showed in *Chytridium olla*, and has been transformed into a zoosporangium. Figure 108 shows such a tube which is exceptionally long measuring 134μ. It is also to be noted here that the zoospores formed in the tip failed to emerge and have germinated *in situ*. Quite often resting spore germination may be arrested so that the incipient sporangia degenerate leaving a large amount of refringent material, as in figure 109 which shows a spore having a long tube, but no further development.

The method of resting spore germination described above is fundamentally similar to that reported for most chytrids. The resting spore functions as a cyst or prosporangium and upon germination gives rise to a thin-walled evanescent zoosporangium as in *Rhizidium mycophilum* (Nowakowski, 1877a), *Polyphagus Euglenae* (Nowaskowski, 1877b); (Wager, 1913), *Entophlyctis vaucheriae* (Fisch, 1884), *Chytridium olla* (De Bary, 1884), *Megachytrium Westonii* (Sparrow, 1933), *Chytridium lagenaria* (Karling 1936b), *Diplophlyctis intestina* (Karling 1936a), *Rhizidiopsis Emmanuelensis* (Sparrow 1933, 1936), *Rhizophidium graminis* (Ledingham, 1936) and *Rhizophidium sp.* (Karling, 1939).

The nature of the refringent substance of the zoospores and resting spore.—One of the characteristic features of many chytrid zoospores is the presence of a spherical or oval, highly refractive globule. This substance has many of the properties of oil, and it has been commonly described in the literature as an oil or fat droplet. This body in *Endochytrium* and in most other rhizidiaceous chytrids is usually spherical, and its plastic and viscid nature becomes evident during the amoeboid stages of the zoospores. Its composition is apparently more complex than that of a simple fat or fatty acid and until further specific chemical tests have been made to determine its nature I shall use the descriptive term "highly refractive" or "refringent globule."

As has been noted above, the highly refractive substance occurs most abundantly in the resting spore, forming a large central globule, and several of the common tests for fats and oils have been made on the substance within the spore. By soaking the resting-spore in sodium hypochlorite for two hours the thick walls were dissolved to permit rapid penetration of the reagents and to facilitate observations. There is the possibility that the treatment thus given the resting spores in dissolving the cell walls may have changed the composition of the cell contents, but in each case the results of the tests with the solvents and stains were the same as those of the untreated spores.

The substance in the resting spore is soluble in ether, chloroform, xylol and insoluble in absolute alcohol at room temperature. It reduces osmic acid and is stained by the Sudan III, Sudan IV and Nile blue sulfate, which are specific for fat. Sudan III and IV stain the highly refractive substance yellow to scarlet, and Nile blue sulfate stains the substance either red, blue, or reddish blue thus making it impossible to determine whether the material is a neutral fat or a fatty acid. Sudan stains should give a scarlet red color with fats, but at times a yellow color was obtained. The

highly refractive substance in degenerating protoplasm, such as that shown in the sporangium of figure 109 stains much more rapidly and brilliantly than the substance found in living protoplasm. This, according to Cowdry (1924) may be due to the physiological condition of the organism, the yellow color resulting when staining a living cell and red when the cell is dead. The substance does not give any of the glycogen reactions with either iodine-potassium iodide, Best's carmine method, or by hydrolysis to form sugar.

The fat indicators Sudan III, IV and Nile blue sulfate were applied to the fungus in all stages of its development. The results indicate that the highly refractive substance is present at all times in various stages of dispersion, and particularly abundant in the rhizoids during the developmental stages. In the rhizoids of mature evanescent sporangia it has been observed only in the distal positions which would indicate that most of the highly refractive substance has been transferred to the sporangium.

DISCUSSION

The Chytridiales are not represented by any single type of thallus structure. The simpler forms included in the Myxochytridineae are holocarpic and without absorbing organs. The Rhizidiaceae, on the other hand, are eucarpic with an absorbing organ that varies from a simple unbranched knob, peg, or filament as in *Tylochytrium* to a well-developed and extensive rhizoidal system as in *E. operculatum* and *Rhizophlyctis petersenii*. The Cladochytriaceae, which include more highly developed forms, possess a rhizomycilium with many centers of growth and reproduction. The thallus of the Rhizidiaceae may thus perhaps be regarded as an intermediate type between the nonrhizoidal and rhizomyceloid species. This variation in the structure and complexity of the thallus has suggested many problems dealing with the possible evolutionary tendencies of this group in relation to the origin of the mycelium and the nature of the thallus organization with respect to nuclear distribution. Heretofore, the Rhizidiaceae have been considered largely from the standpoint of sexuality and phylogeny, based mainly upon the study of living material, and few studies have been made on fixed and stained material with respect to the morphology of the thallus.

In this study of *E. operculatum* it has been observed that the nucleus, upon germination of the zoospore, migrates into the incipient sporangium thus transferring the center of growth and reproduction. During the developmental stages, the thallus consists of a single cell which is modified

locally into a vegetative center of growth and a rhizoidal system. Although evidence of flow of protoplasmic material between the rhizoid and the incipient sporangium has been found, nuclei have not been observed in the rhizoids but appear to be localized within the developing sporangium. Nemec (1912) had found that nuclei in the thallus of *Entophlyctis salicornae* were localized in the sporangium but, as far as I am aware this is the only report concerning nuclear distribution in an intramatrical, rhizidiaceous form possessing a well developed rhizoidal system. The localization of the nuclei in the incipient sporangium of *E. operculatum* and their exclusion from the rhizoids precludes the possibility of the reduplication of centers of growth and reproduction within the absorbing system. Karling (1937b) from his study of *Cladochytrium replicatum* concluded that the reduplication of centers of organization was associated with the migration of one of the daughter nuclei following the first division of the primary nucleus in the spindle organ into the rhizomycelium. At this time he suggested that the development of the monocentric type of thallus possessing rhizoids is associated with the localization of nuclei in the sporangium and that additional centers of growth and reproduction are not found since the nuclei do not migrate into or occur in the rhizoids. This investigation of *E. operculatum* tends to support the contention of Karling regarding the nature of thallus organization.

The question of differentiation of the absorbing organ of the Rhizidiaceae from a mycelium, holdfast, rhizomycelium and possibly a rhizoid is yet a matter that must be settled. Zopf (1884), Schroeter (1897) and Fitzpatrick (1930) consider the rhizoids as mycelia, while Fischer (1892), Atkinson (1909), Petersen (1910), and Gauman and Dodge (1928) call them rhizoids. Bessey (1935) uses either rhizoid or haustorium, while Nemec (1912) used the term haustorium. The terminology employed for the vegetative and absorbing organs of the fungi was reviewed by Karling (1932) in which he defined rhizoid as being distinguishable from the mycelium by "their decreasing in diameter from the point of origin and tapering to extreme fineness without any tendency to form new centers for the processes of growth differentiation and reproduction." It is distinguished from the haustorium by its tapering to a fine tip instead of remaining as broad lobes as the haustoria. Its function is different too in that the rhizoid is usually present in saprophytic forms while the haustorium is associated with parasitic species. It appears from the evidence obtained in this study that rhizoids may be further distinguished from the mycelium and rhizomycelium by their lack of nuclei.

It is significant to note here that the nuclear cap in *E. operculatum* is strikingly similar to that found in species of the Blastocladiales, but whether or not this is indicative of relationship is still uncertain. At present the presence of the nuclear cap in the zoospore of the Myxochytridineae described and the references to it in the Rhizidiaceae are rather vague, although it appears to be present in the latter family. The figures of the zoospore of *Polyphagus Euglenae* show that there is a deeply staining mass of material about the nucleus, which Wager (1913) explains as a chromidial mass, and it appears to be much like the nuclear cap in *E. operculatum*. In other species of the family which have been studied to some extent from fixed and stained preparations, namely *Zygorhizidium Willei*, Lowenthal (1905), *Mitochytridium ramosum*, Couch (1935b) and *Rhizophidium Beauchampi*, Hovasse (1936), there is a suggestion of nuclear caps, but the figures and descriptions are as yet insufficient. The nuclear cap is, however, well defined in *Blastocladia Pringsheimii* (Thaxter) (1896), *B. strangulata* (Barrett 1912), B. Pringsheimii and B. Globosa (Cotner 1930a), *Allomyces Javanicus* (Kneip 1929), *A. arbuscula* (Hatch 1935, 1938). Cotner (1930b) shows structures in the gametes of *Apodachlya brachynema*, *Saprolegnia monoica* var. *glomerata*, *Achlya conspicua*, *Aphanomyces euteiches* and *Phytophthora palmivora* which may be nuclear caps, but are less sharply defined than those in the preceding group. The structure of the zoospore has been utilized by Miss Matthews (1937) as a criterion to establish *Blastocladiella*, a new genus, with the Blastocladiales rather than to the Chytridiales although in thallus structure it resembles *Macrochytrium botrydioides* of the Rhizidiaceae more closely. If the zoospore structure of *E. operculatum* is compared with that of other species of the Phycomycetes it becomes evident that outside of the Rhizidaceae it is more closely allied to the *Cladochytrium replicatum* and the Blastocladiales than to the Myxochytridineae.

SUMMARY

The cytology of *E. operculatum* is described in its relation to the development and organization of the thallus, the formation of the evanescent sporangium, and the development and germination of the resting spore.

The zoospore possesses an extra-nuclear cap which appears to vary greatly in form and in its staining reactions. The origin and function of the cap have not been determined.

Upon germination the zoospore produces a filamentous branched or unbranched germ tube, into which the nucleus migrates. The latter comes to rest in a swollen portion of the germ tube which becomes the new center of organization of the thallus. The distal portions of the germ tube give rise to the rhizoidal system.

The nucleus following its arrival in the rudiment of the sporangium enlarges, probably doubling its size before division occurs. Subsequently enlargement of the sporangium is accompanied by nuclear division.

Nuclear division in the primary evanescent sporangium is mitotic and simultaneous. The spindle is intra-nuclear with central bodies at the poles which appear to be extended through the nuclear membrane during the anaphases. Astral rays have been found to radiate from the central bodies of some nuclei. Cleavage of the protoplast to form the uninucleate zoospores is by progressive furrowing.

The development of the resting spore is similar to that of the evanescent sporangium up to a certain stage, but they develop a thick, rough or smooth wall which appears to be composed of three layers. The protoplast is displaced to the wall of the resting spore as a thin peripheral layer by the large highly refractive globule that occupies the center of the cell. The spore, usually uninucleate, may occasionally be bi-nucleate.

The resting spore functions as a prosporangium and upon germination develops an operculate evanescent sporangium on its surface or at the end of a long tube.

The center of growth of the *E. operculatum* is transferred from the zoospore to some position in the germ tube which then becomes the zoosporangium. The thallus is monocentric and this type of organization appears to be associated with the localization of nuclei in the zoosporangium and their exclusion from the rhizoids.

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Stem Morphogenesis in Lycopersicum: A Quantitative Study of Cell Size and Number in the Tomato

HELEN B. HOUGHTALING

(WITH PLATE 8 AND TEN FIGURES)

In a typical dicotyledenous herbaceous plant, the stem increases in length as the result of the activity of a group of meristematic cells situated at its tip. By these cells the primary tissues are laid down and the fundamental anatomical arrangement of the stem is determined. For several internodes below this meristem, growth takes place in the primary tissues at different rates with the result that the relative sizes of the tissues are changed (Sinnott, 1936). Thus the anatomical pattern is altered. The purpose of this study is to determine what changes take place in the development of the stem in several varieties of *Lycopersicum esculentum* and one variety of *L. pimpinellifolium*, and to discover the mechanism through which the differences in stem size which exist in these types and their hybrids become established.

One of Mendel's original seven characters in peas was concerned with length of stem. Dwarf plants were recessive to tall, and segregated in the F_2 in the ratio of one dwarf to three tall. The inheritance of height in peas was found to be more complicated by Keeble and Pellew (1910) who found two factor pairs concerned with length and thickness of stem respectively. These combined to make a very tall plant. The double recessive produced an extreme dwarf type, and either factor alone produced a plant which was intermediate between the two extremes. Numerous examples of genetic control of height in plants might be cited. In tomatoes the dwarf habit of growth is recessive to the tall, and is controlled by a single pair of Mendelian factors. Thus we have direct evidence that length of stem and height of plant are genetically controlled not only in other plants but in the tomato itself.

The work of Sinnott, Blakeslee and Houghtaling (1934) on *Datura* peduncles offers an excellent example of the anatomical structure of the plant being subject to genetic control. In this genus the presence of an extra chromosome affects both anatomical structure and cell size. Except within the polyploid series, there is little evidence for a necessary relationship between anatomical pattern and size of stem. Large stems may be large through increase in number of cells or through increase in size of cells. Sometimes vascular tissue may be increased without the increase necessarily being accompanied by any other structural enlargement.

However, work on *Datura* was all done at the midpoint of the flower peduncle on the day of flowering. Work has been done on the developmental relationship of cell size to fruit size (Houghtaling, 1935; Sinnott, 1939), and it has been found that, in general, a period of cell division with perhaps some increase in cell size is followed by a period of cell enlargement with no further division. This present study deals with cell and tissue relationships in the development of the main stem of the tomato.

MATERIAL AND METHODS

The material used was derived from three pure lines of *Lycopersicum esculentum*, "Bonnie Best," "Red Cherry," and "Dwarf Champion," and one pure line of *L. pimpinellifolium*, "Red Currant." These had all been inbred through five to eight generations and showed no recognizable genetical variability. Crosses were made between Bonnie Best and Red Cherry, Bonnie Best and Red Currant, and Dwarf Champion and Red Currant. One F_2 of one hundred plants was grown from the F_1 of the cross Bonnie Best by Red Currant. All these plants were grown in the University of Michigan Botanical Gardens during the summer of 1935. More were grown in 1936 and 1937, but the material studied was largely taken from the plants raised in 1935. The photographs (plate 8) were taken in 1937, but comparable measurements showed that these plants were essentially similar to those raised in 1935.

These particular types were chosen for study because they were characterized by large differences in thickness of stem (plate 8). The difference between *L. pimpinellifolium* and *L. esculentum* is, as might be expected, the greatest. Although the various types of *L. esculentum* are vegetatively very similar, still an obvious difference exists between Bonnie Best and Dwarf Champion.

Certain gross measurements were made in the field. Stem and internode length were measured with a ruler, and diameters were measured by micrometer calipers. For all length measurements the meristem was taken as the point of reference. A few mature fruits were measured from

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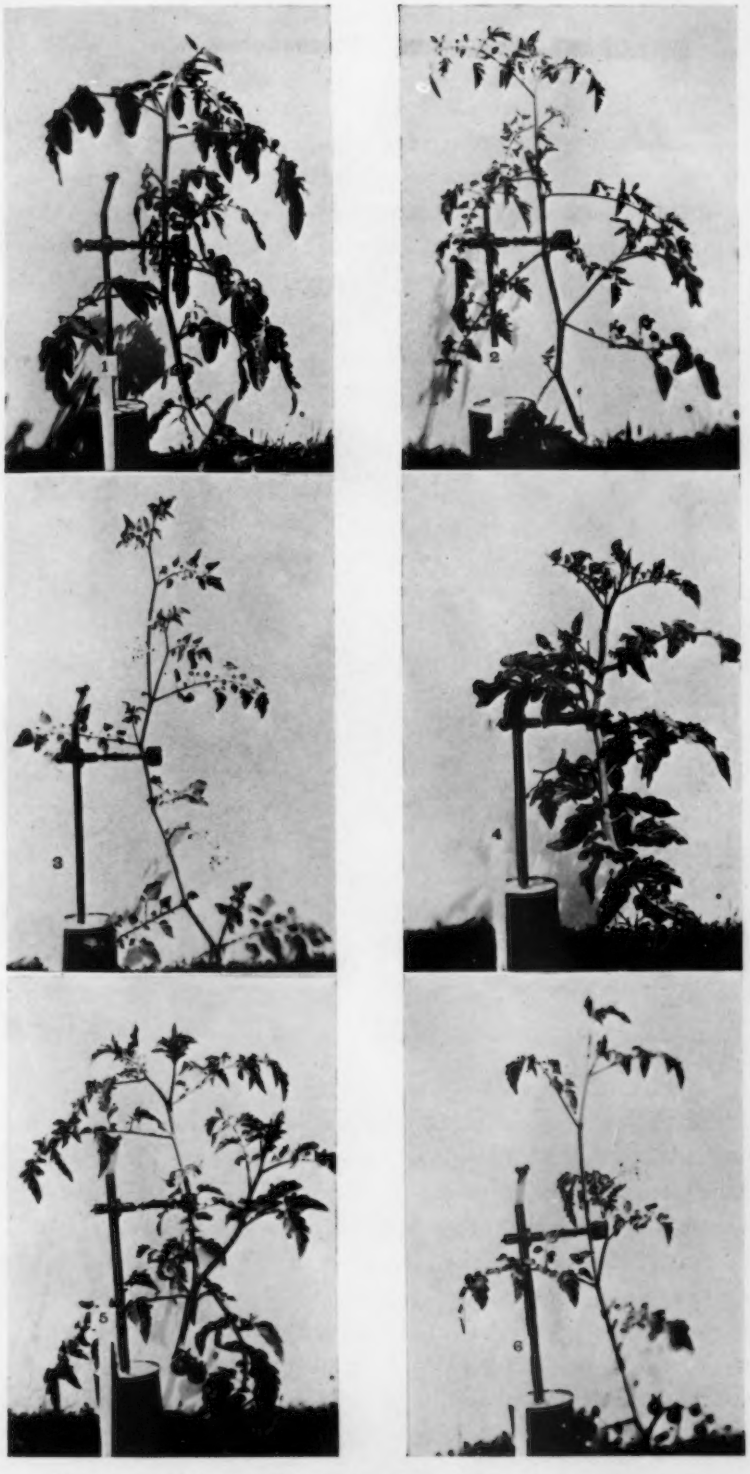
Explanation of Plate 8

Branches from typical plants

1 = Bonnie Best
2 = Red Cherry
3 = Red Currant
4 = Dwarf Champion
5 = Red Cherry × Bonnie Best
6 = Dwarf Champion × Red Currant


1
2
3
4
5
6

every plant. The fruits were cut along a polar axis, and the polar and equatorial diameters were measured with a ruler.

For anatomical study, transverse and longitudinal hand sections were made along the stem at various distances from the meristem. These were stained in safranin and mounted for study in glycerin. For more careful study, material was fixed in seventy percent alcohol, with five percent

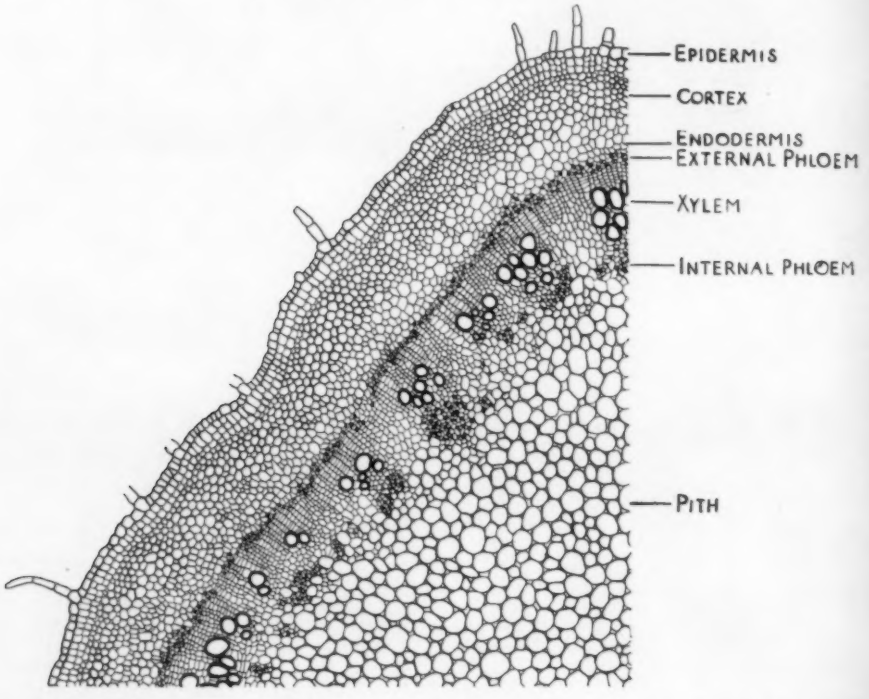


Fig. 1. Diagram of a portion of tranverse section through a stem of Bonnie Best showing tissues studied.

acetic acid and five percent formalin added, then imbedded in paraffin, sectioned with a rotary microtome ten to fifty micra thick, and stained in Delafield's haematoxylin and safranin.

The anatomy of the typical tomato stem has been described by Woodcock (1936). Externally the stem is protected by a single epidermal layer (fig. 1). Underneath this is a hypodermal layer which in young stems is one cell thick. This single layer of cells apparently divides, forming two or three layers in older stems. The outermost cells frequently elongate radially. These cells contain chloroplasts. Beneath the hypodermis there is a region of parenchymatous cells which form

the major part of the cortex. An endodermal layer is marked by the presence of starch grains when fresh material is stained with iodine. Woodcock was able to discern Casparian strips, but the author could find none in this material. The cross section of the vascular bundle shows both internal and external phloem, lying in patches on either side of the xylem. The cambium is easily visible, and in older stems considerable secondary growth is evident. Within the ring of vascular tissue lies the pith made up of large parenchymatous cells.

Anatomical measurements were made in various ways, some with a micrometer eyepiece in the ordinary compound microscope, but the majority with a projection apparatus. The sections were put in the machine, the magnified image was projected on paper and drawn, and measurements were made on the drawings. Various magnifications were used, care being exercised that all measurements of a given kind were made at the same time. In measuring cells the ten largest were chosen from each tissue. This was done to avoid those which were not cut through the equatorial diameter and to standardize procedure as much as possible. Ten cells were measured from typical regions of the epidermis, cortex, and pith. In cortical and pith cells, two diameters of each cell were measured at right angles to one another and averaged. The average was recorded as representing the diameter of the cell.

To calculate the cross-sectional area of the cell, this diameter was squared and multiplied by the factor $\Pi/4m,^2$ where m denotes the magnification. This gave the area of a circle with the original diameter. Thus it was possible to correct for shape and magnification with a single mathematical operation. Since the epidermal cells were not circular in shape it was necessary to multiply the two diameters obtained in the original measurements together to obtain the area of a rectangle and then correct for magnification. These figures, circle and rectangle, closely approximate the actual shape, and hence also the area, of the cells concerned. Further, since the variations from these fundamental figures are at random within a tissue, these calculations may safely be used for the purpose of comparison.

To find a representative cell size for each tissue, the geometric mean of the area of the ten cells measured was calculated. The reason for using geometric means rather than ordinary (i.e., arithmetic) averages lies in the fact that these measurements are of growing cells and growth is a geometric process. Likewise, by the method of computation of cross-sectional areas which was used, the geometric mean was more easily

obtained. In any case the geometric mean differs only slightly from the arithmetic mean and seems to give a more accurate picture of size changes.

Tissue measurements were made from the same sections in which cell size was studied. The stem was projected at a lower magnification and drawn. A line was drawn at the outer limit of the epidermis, one at the outer limit of the external phloem, and one at the inner limit of the internal phloem. Two diameters of each were measured. The area of each was calculated from the average diameter in the same manner as area of pith cells was found. From these areas the cross-sectional area of the stem, cortex, vascular cylinder, and pith were computed.

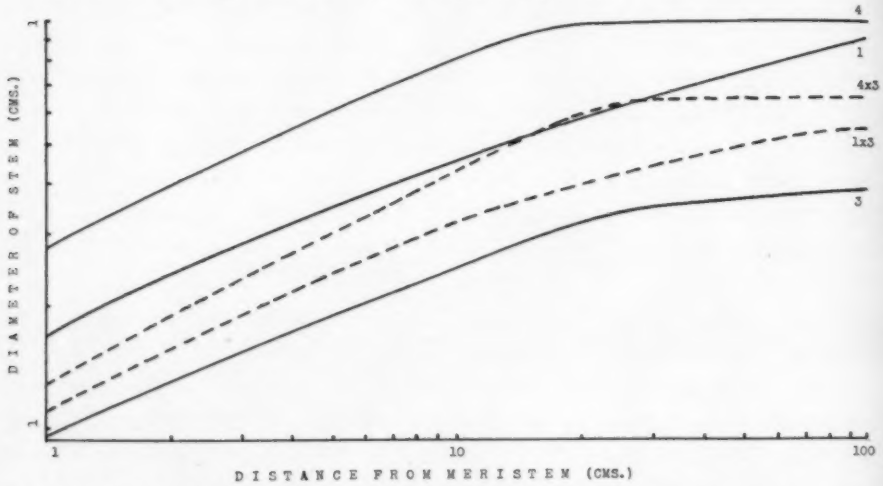


Fig. 2. Diameter of stem plotted logarithmically against distance from the meristem. Each curve represents approximately 100 measurements.

4 = Dwarf Champion **1 = Bonnie Best**
4 × 3 = Dwarf Champion × Red Currant
1 × 3 = Bonnie Best × Red Currant

RESULTS

The differences in diameter of stem and length of internodes are shown in the photographs (plate 8). Measurements show that at equal distances from the stem tip these various tomato varieties have marked differences in diameter. Figure 2 shows the diameter of the stem measured at the midpoint of the internode plotted logarithmically against the distance of that point from the tip of the stem. In the pure lines the difference in stem diameter is already established at one centimeter from the tip. Measurements with calipers were not very accurate within a distance of one centimeter from the tip because of the proximity of young leaves. However,

examination of microscope sections shows that the differences in stem size continue back into the meristem itself. The curves in figure 2 show that the differences which are established so early are maintained throughout the growth of the stem. For example, at one centimeter from the tip the stem diameter of Bonnie Best is a little less than twice that of Red Currant, at five centimeters from the tip the former is still just a little less than double the latter, and at ten centimeters the same relationship holds. In other words, relative increase in stem diameter takes place at the same rate with respect to distance from the meristem in both types. Figure 2 shows that this is true for all the types studied. The tendency for the cures to flatten between ten and twenty centimeters from the tip is undoubtedly because of the termination of increase in diameter by primary growth with the assumption of secondary growth. The hybrid, Bonnie Best × Red Currant, lies between its two parents, rather nearer Red Currant.

Table 1 gives the average diameters of the stems at five and ten centimeters from the tip. For the hybrids, the arithmetic and geometric means of the parents are given for comparison with the attained values. It is obvious that the hybrids lie very close to the geometric means. The fact that the values obtained from the measurements are slightly smaller in all

TABLE 1

Diameters of Stems

LINE	DIAMETER 5 CMS. FROM TIP	DIAMETER 10 CMS. FROM TIP
RED CURRANT	.187	.250
BONNIE BEST	.350	.450
DWARF CHAMPION	.595	.800
BONNIE BEST × RED CURRANT	.240	.325
Arithmetic mean	.265	.350
Geometric mean	.256	.335
DWARF CHAMPION × RED CURRANT	.300	.425
Arithmetic mean	.386	.525
Geometric mean	.324	.447

instances suggests partial dominance of the smaller parent. The slightly steeper slope of the hybrid, Dwarf Champion × Red Currant, indicating more rapid relative increase in stem diameter, may be the result of hybrid vigor, although there is no indication of the same effect in the other hybrid.

In the course of this work the author noted that in the F_2 population, in which there was complicated segregation of fruit size (fig. 3), the plants

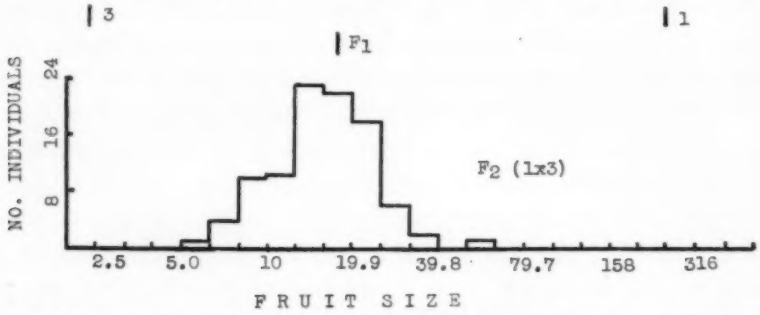


Fig. 3. Diagram shows distribution of fruit size on a logarithmic scale in the F_2 from a cross of Bonnie Best × Red Currant. The positions of the mean value of fruit size in the parents and F_1 are indicated by vertical lines.

with the thicker stems bore larger fruits than those with thinner stems. In the parental types this relation is fairly obvious. The stem diameter of Red Currant at ten centimeters from the tip is .250 cms., and the fruit

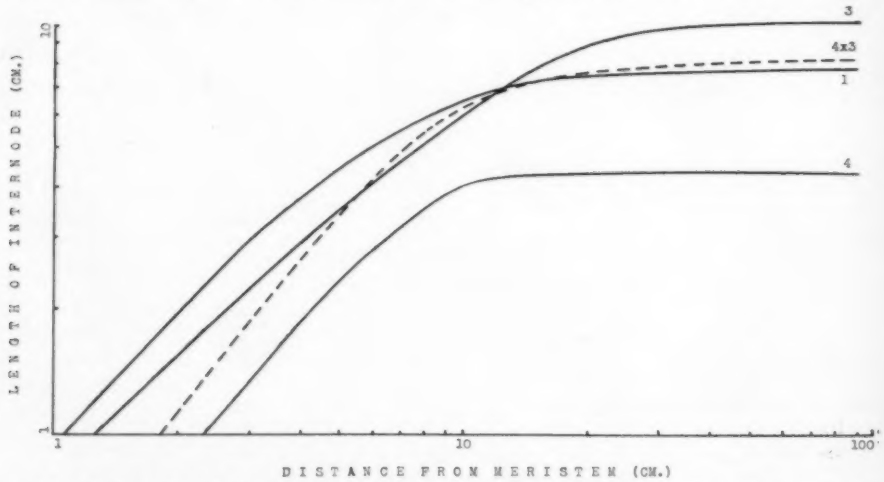


Fig. 4. Length of internodes plotted logarithmically against distance from the meristem. Each curve represents approximately 100 measurements.

3 = Red Currant
4 = Dwarf Champion
4× 3 = Dwarf Champion × Red Currant
1 = Bonnie Best

volume is about 1.5 cu. cm. The corresponding figures for Bonnie Best are .450 cm. for the diameter at ten centimeters and 275 cu. cm. In the F_2 from the cross Bonnie Best × Red Currant, a correlation of .57 ± .03

was found between size of fruit and diameter of stem measured ten centimeters from the meristem. This relation may, of course, be the result of genetic linkage between factors controlling stem and fruit size. It may also be physiological in that small-stemmed types may be unable to bear large fruits. However, since the diameter of the stem is determined in the meristem and Houghtaling (1935) has shown that the size of the fruit is also determined in the fruit meristem, it is suggested that a morphological correlation may exist between the meristems of the plant.

The illustrations (plate 8) indicate little difference in internode length except in Dwarf Champion. Statistical data show that this length is subject to great variability. There seem to be no or only slight differences existing among all the types with the exception of Dwarf Champion (fig. 4). In the latter the internodes are relatively shorter than in the other varieties, not only at first but throughout development so that the relative internode length is maintained. It seems rather surprising that with the great difference in diameter that exists between Bonnie Best and Red Currant there should be practically no difference in length of internodes. The factors which control diameter have no influence upon length.

Another point of some interest which appeared in measuring internode length is the fact that the internode below a branch is generally shorter than the internode above the branch although the latter is, of course, the younger. This is particularly noticeable in the thick-stemmed types. For example, in Bonnie Best the internode below a branch averages 67 percent as large as the internode above the branch; in Dwarf Champion, 79 percent; in Red Cherry, 88 percent; and in Red Currant, 89 percent.

In figure 5 the relation is shown between cross-sectional area of the tissues in the stem and the area of the stem. The distances between the lines indicate the differences in cross-sectional area of a single tissue in the several varieties. The slope of the lines indicate the relative rate of growth of the tissues with respect to stem area, or, more simply, the value of Huxley's constant, "k" (Huxley, 1932).

For the pith the slope of the lines, or "k," is always unity, which indicates that the area of the pith increases at the same rate as the area of the stem. In other words, the area of the pith occupies the same proportion of the stem throughout development. The differences in position of the lines show that relatively more of the stem is made up of pith in Dwarf Champion than in Bonnie Best, and more in Bonnie Best than in Red Currant. The hybrid between these last two lies intermediate between them.

The fact that these lines do not converge at the base indicates that the differences in size of pith are initial differences determined in the meristem and maintained as such through development. It is interesting to note (cf. table 2) that the area of the pith in the types with larger stems is proportionately greater than in those with smaller stems.

For cross-sectional area of vascular tissue the story is not quite so simple. The slopes of the lines are different, being greater for Red Currant than for Bonnie Best, with the hybrid lying between. Dwarf Champion has the least slope of all. This is, of course, in the reverse order of stem size. There is a tendency for the lines to converge at the base. Thus if Dwarf Champion ever produced a stem as small as the smallest studied of Red Currant it would have approximately the same amount of vascular tissue. However, the rate of increase of vascular tissue in Red Currant is much greater than in Dwarf Champion with the result that ultimately the small-stemmed type has a much larger proportion of vascular tissue. The rate of increase of cross-sectional area of vascular tissue with reference to cross-sectional area of the stem is inversely proportional to the typical stem thickness of the varieties studied (table 2).

TABLE 2

Cross sectional areas of Stems and Huxley's Constant, k

LINE	CROSS-SECTIONAL AREA OF STEM AT 10 CMS.	PERCENT PITH	K VASC. CYL. AND STEM	K CORTEX AND STEM
RED CURRANT	.0492	30	1.26	.77
BONNIE BEST	.1580	40.5	1.10	.84
DWARF CHAMPION	.5120	49	1.05	.87
BONNIE BEST × RED CURRANT	.0860	39	1.14	.80

In the pith the slope of the lines for all types is 1. In the vascular cylinder the slope, or "k," is always greater than 1. The area of the vascular tissue in the cross-section increases faster than the stem as a whole. Therefore the cortex cannot increase as fast as the area of the

Explanation of Figure 5

Fig. 5. Cross-sectional area of cortex, vascular cylinder, and pith plotted logarithmically against cross-sectional area of stem.

	NO. OF SECTIONS
1 = Bonnie Best	32
4 = Dwarf Champion	17
3 = Red Currant	34
1 × 3 = Bonnie Best × Red Currant	23

The curve showing the relation of the cortex to the stem area for the hybrid was omitted because it lay too close to the curve for Red Currant to show distinctly.

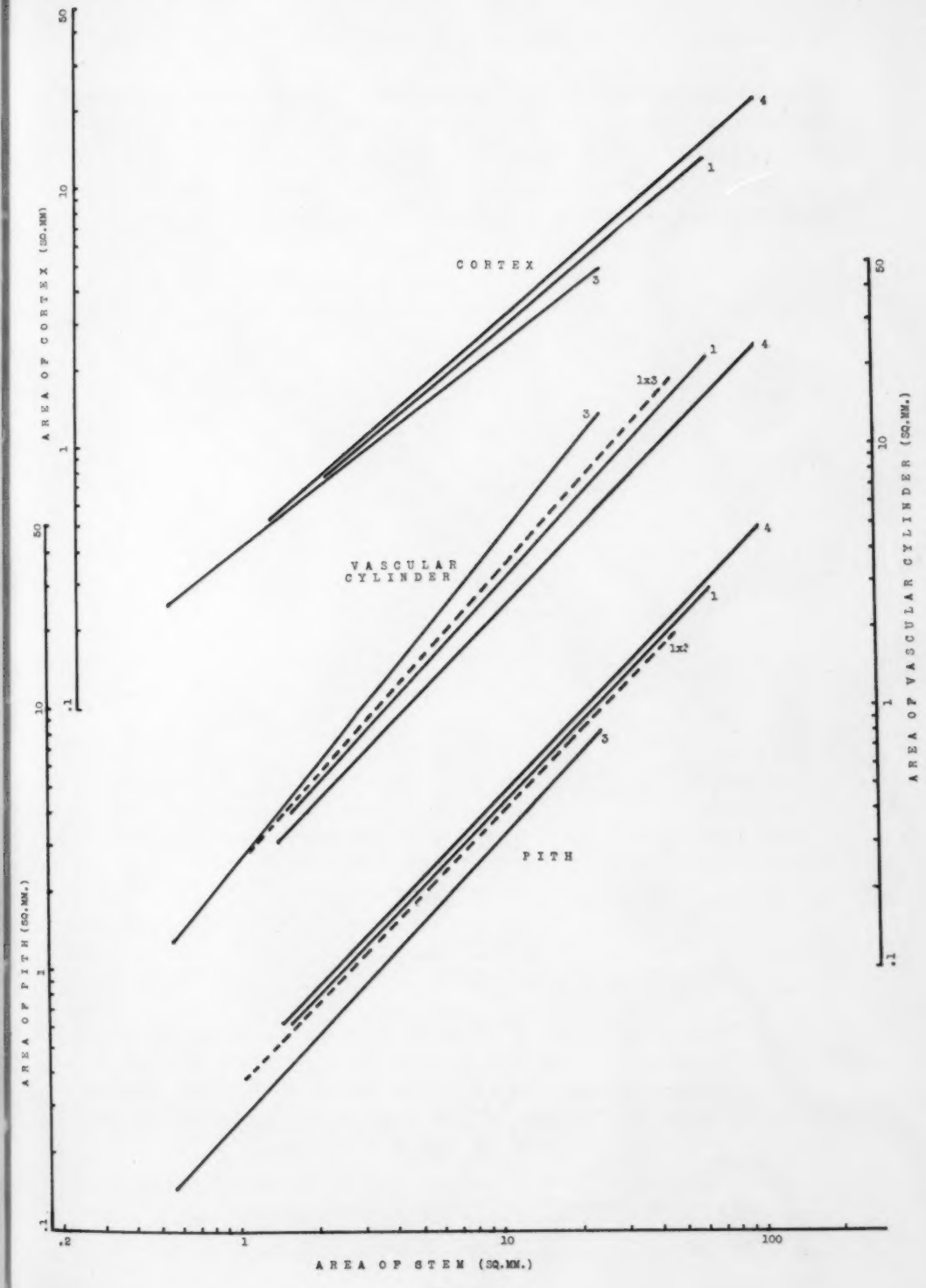

CORTEX
VASCULAR CYLINDER
PITH
1x3
1x2
AREA OF CORTEX (SQ.MM)
AREA OF VASCULAR CYLINDER (SQ.MM.)
AREA OF PITH (SQ.MM.)
AREA OF STEM (SQ.MM.)
50
10
1
.1
.2
100

Figure 5

stem. Also since the greatest rate of increase of vascular tissue is found in Red Currant, it is obvious that the slowest rate of increase in the cortex must be found in Red Currant. The lines in figure 5 and the values of "k" for relative rate of increase of cortex with respect to stem in table 2 show that this expectation is fulfilled. Whereas the rate of increase of

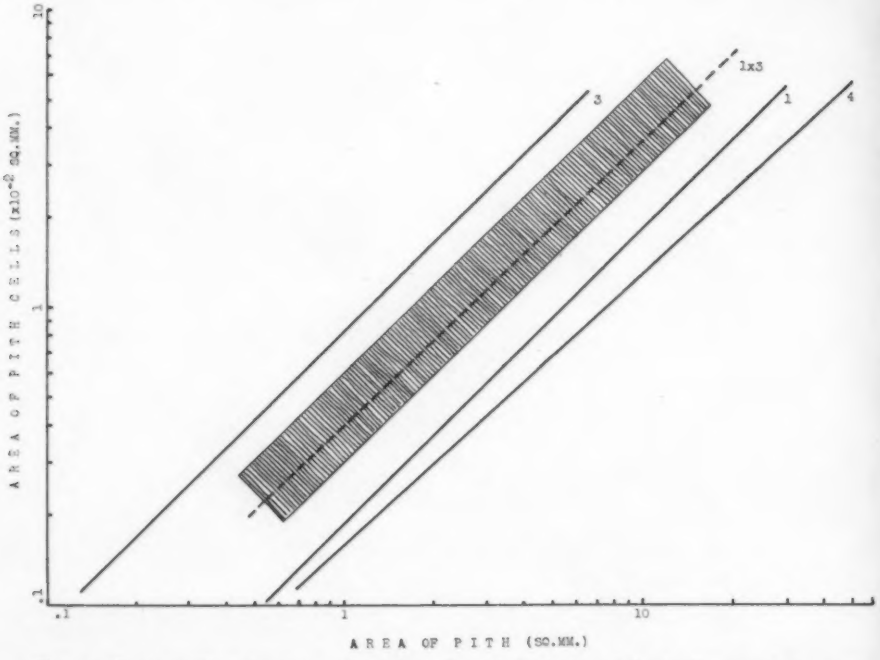


Fig. 6. Cross-sectional area of pith cells plotted logarithmically against cross-sectional area of pith. The shaded area shows the range of the F_2 curves.

	NO. OF CELLS MEASURED
4 = Dwarf Champion	170
1 = Bonnie Best	320
3 = Red Currant	340
1 × 3 = Bonnie Best × Red Currant	170

vascular tissue increases inversely with the stem size of the various types, the rate of increase of cortical tissue varies directly with the stem size.

Cell size was compared with tissue size. Figure 6 shows the relation between cross-sectional area of pith cells with cross-sectional area of pith. The slope of the lines, or "k," is approximately 1, and the cells increase in cross-sectional area at the same rate as the pith. Evidently cell division has stopped and increase in pith area is accomplished by increase in cell size. This is true in all types studied. However, for a pith of given size,

large differences in cell size appear. Red Currant, the smallest-stemmed type, has the largest pith cells. Dwarf Champion, the largest-stemmed type, has the smallest cells, and the other kinds are intermediate in order of their stem size. Figure 7 shows that the same condition holds for cortical cells. The differences in cell size, both initial and ultimate, are smaller than in the pith, however.

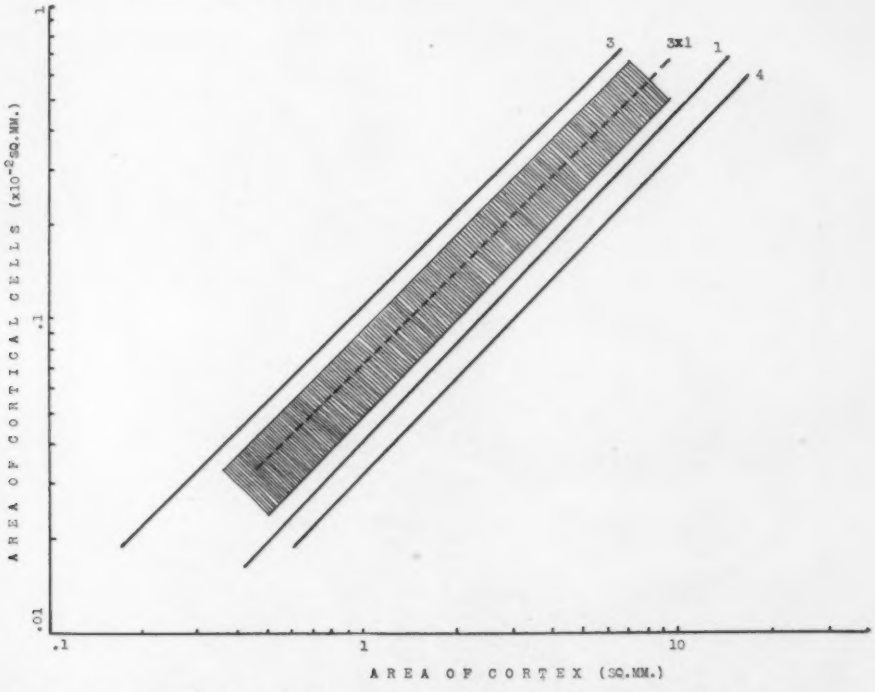


Fig. 7. Cross-sectional area of cortical cells plotted logarithmically against cross-sectional area of cortex. The shaded area shows the range of the F_2 curves.

	NO. OF CELLS MEASURED
4 = Dwarf Champion	170
1 = Bonnie Best	320
3 = Red Currant	340
1 × 3 = Bonnie Best × Red Currant	230

The cross-sectional area of the epidermal cells is compared with the cross-sectional area of the stem (fig. 8). This relation is probably not a causal one, but it does indicate that the size of the cells does not increase as rapidly at first as the size of the stems, which means that for some time cell division continues to occur. Later the slope of the curve does approach 1, at which time cell division has stopped. It has long been known (Brotherton and Bartlett, 1918; Sinnott, 1930) that cell division may persist

longer in the epidermal cells than in the other primary tissues. Most interesting is the fact that the order of the curves shows that the same relative cell size and tissue or stem relationship holds here that exists in the other tissues. Red Currant, the smallest-stemmed type, has comparatively the largest cells in all tissues, with the hybrid Bonnie Best × Red Currant next, then Bonnie Best, and finally Dwarf Champion. Thus the larger the stem of a variety, the smaller its cells are with respect to those of the other varieties.

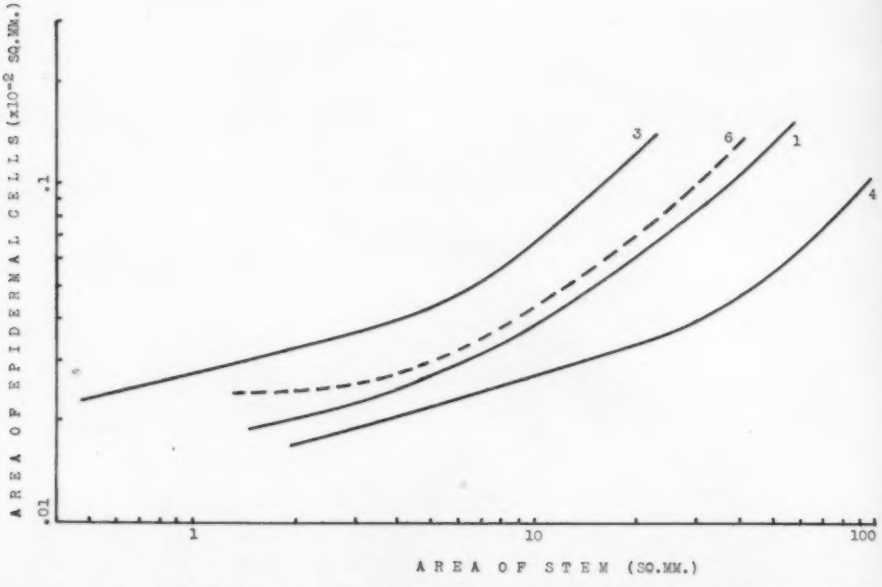


Fig. 8. Cross-sectional area of epidermal cells plotted logarithmically against cross-sectional area of stem. The number of cells measured for each curve varies from 170 to 370.

3 = Red Currant
1 = Bonnie Best
4 = Dwarf Champion
6 = Dwarf Champion × Red Currant

In a pith with cross-sectional area of one square millimeter, Red Currant will have much larger cells than Bonnie Best will have in pith of equal size. But Bonnie Best starts development with a much larger pith than Red Currant and therefore pith area of one square millimeter will be found in much younger stem of Bonnie Best than in Red Currant. Thus the possibility of a relationship between cell size and age, or, perhaps more suitably, degree of maturation, is suggested. The degree of maturation, a function of time, can be obtained only through some indirect measurement. The measure which suggested itself was the distance from the

meristem. Figure 9 shows the relation to distance from the meristem of stem diameter, pith diameter, thickness of vascular tissue, and diameter of pith cells, for one individual stem from a plant each of Red Currant and Bonnie Best. These are all plotted on a logarithmic scale to the base

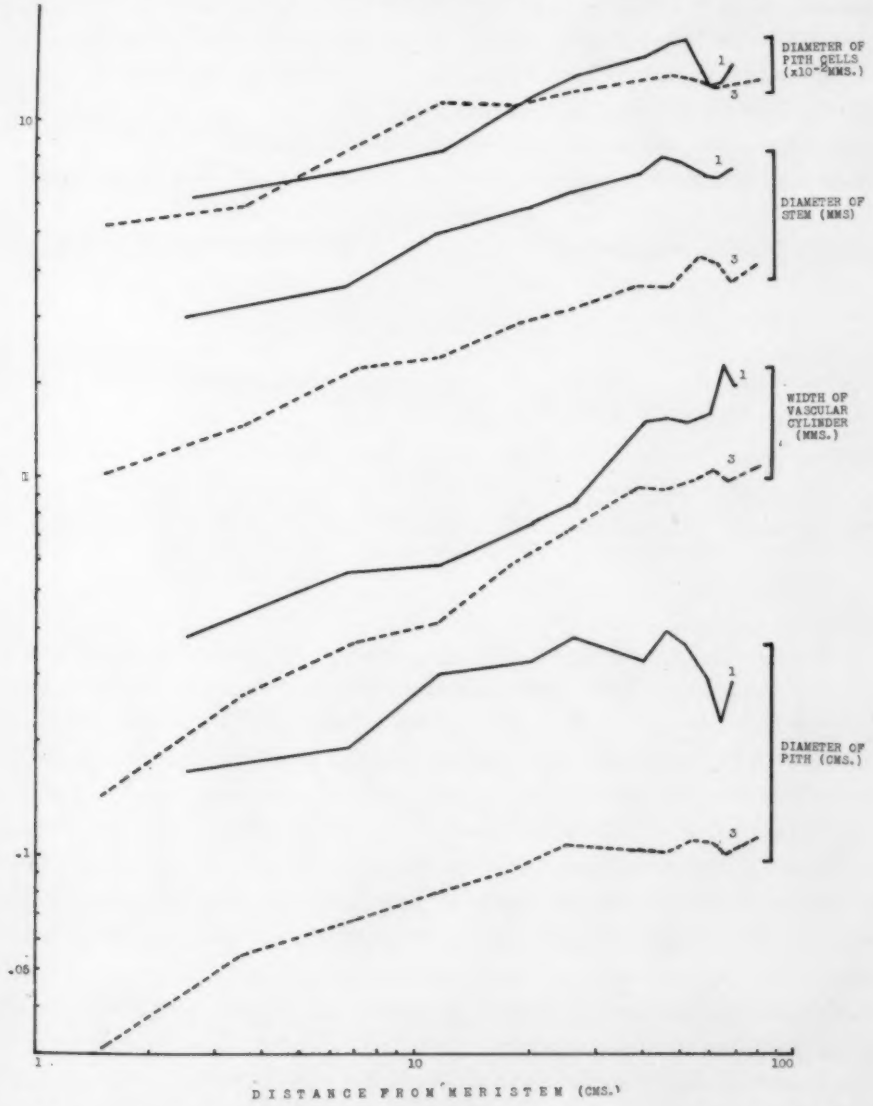


Fig. 9. Diameter of pith cells, stem, pith, and width of vascular tissue plotted logarithmically against distance from meristem.

1 = Bonnie Best 3 = Red Currant

ten with equal intervals representing the multiple ten (as, for instance, those from 1 to 10 and from 10 to 100, respectively). Thus the distances between the curves for Bonnie Best and Red Currant for stem diameter may be compared directly with the distances between the curves for pith cells.

With this in mind it is obvious from the figure that although great differences in size of pith exist, there is no difference in pith cell size among the varieties with long internodes if distance from the meristem is the same. Also, the size of cortical cells is exactly parallel, and is strictly a function of distance from the meristem. This is further brought out in table 3 where cell size is given at various intervals down the stem. There is very close agreement for Bonnie Best, Red Currant, and their hybrid.

TABLE 3

Cross-sectional Area of Cells

LINE	DISTANCE FROM MERISTEM							
	1 cm.		2.5 cm.		5 cm.		10 cm.	
	Pith	Cortex	Pith	Cortex	Pith	Cortex	Pith	Cortex
BONNIE BEST	.160	.0295	.39	.062	.71	.104	1.20	.167
RED CURRANT	.165	.0359	.37	.062	.74	.103	1.27	.162
BONNIE BEST × RED CURRANT	.180	.0375	.37	.060	.88	.100	1.32	.165
DWARF CHAMPION	.485	.0620	1.12	.137	1.95	.270	3.60	.400

Bonnie Best and Red Currant are classified as belonging to different species; the former, *Lycopersicum esculentum*, and the latter, *L. pimpinellifolium*. Lindstrom and Humphrey (1933) found that *L. pimpinellifolium* has smaller chromosomes (although the number is the same) than *L. esculentum*. Navashin (1931) working on *Crepis* found a direct relation between amount of chromosomal material and cell size in the meristem. In these two species of tomato, cells were measured in longitudinal sections of the meristem with an eyepiece micrometer and no difference was found in area. The method of making measurements was not as accurate as that employed by Navashin, and it is possible that a more refined technique would show differences. However, in view of the results in older tissues, differences do not seem probable.

From table 3 it is apparent that the cells in the pith (or in the cortex) of Bonnie Best and Red Currant and their hybrid agree almost exactly in size at equal distances from the tip of the stem. This is not true in Dwarf Champion in which the cells are very much larger. In Bonnie Best and

Red Currant internode length was the same, but in Dwarf Champion the internodes were shorter. It was thought that perhaps at an equivalent number of internodes from the tip cell size in Dwarf Champion would be the same as in the other types. In making measurements the number of internodes was not counted. However, since it was known that the internodes in Bonnie Best are approximately twice as long as in Dwarf Champion, it might be assumed that at 2.5 centimeters from the tip Dwarf Champion would have as many internodes as Bonnie Best at 5 centimeters from the tip, etc. It is obvious from the table that the cells in Dwarf Champion are still much larger than would be expected on this assumption. However, length from the tip in the morphologically similar varieties and their hybrid was conceived to be an indirect measure of a time interval, and there is no evidence that number of internodes in morphologically different varieties can be treated as a measure of time any more than distance from the meristem.

It has already been pointed out that cell size in the pith and in the cortex, as measured by cross-section, increased at the same rate as tissue. This would indicate that no cell division occurred in these tissues below the meristem. In order to check this conclusion, the cross-sectional area of the tissue was divided by the area of the cells and thus cell number was computed. Although there was considerable variability within each variety, there was no evidence that such variability was not completely at random. Attempts to correlate cell number with tissue cross-section within a variety showed that no relation existed at all and the conclusion was definitely reached that cell number within a variety does not increase below the meristem.

The individual plants of the F_2 from the cross Bonnie Best $\times$ Red Currant were examined developmentally in the same way that the parents were. Fundamentally the same relations held. Cell cross-section increased at the same rate that tissue cross-section did, etc. The group of curves (See figs. 6 and 7), each representing a single individual, fell between the curves for the parents. As might be expected they showed a wider spread than equivalent curves for the parents or F_1, indicating greater variability and thus, as usually interpreted, multiple factor inheritance. There was no clear-cut segregation. These curves were rather difficult to deal with inasmuch as the variability in one individual frequently threw it into the size class of individuals on either side. A large number of measurements would be necessary to locate the curves accurately, and the additional information which would be obtained did not seem sufficient to war-

rant the effort. However, since cell number is fairly constant in the various cross-sections of the individual, it offered a fairly simple means of comparing parents and offspring for that characteristic. The results are given in figure 10 and in greater detail in table 4.

TABLE 4

Comparison of Cell Numbers of Parents and Offspring

		PITH CELLS		CORTICAL CELLS		NUMBER OF CELLS MEASURED
		Number	Standard deviation	Number	Standard deviation	
PURE LINES	BONNIE BEST	563	34	2430	162	320
	RED CHERRY	450	20	2070	169	370
	RED CURRANT	127	5	1045	66	340
	DWARF CHAMPION	664	40	2910	107	170
F_1	RED CHERRY × BONNIE BEST	425	30	1725	128	200
	BONNIE BEST × RED CURRANT	248	14	1410	67	230
F_2: BONNIE BEST × RED CURRANT	1	187	13	1060	88	
	3	193	12	1210	121	
	5	149	10	1160	106	
	6	187	15	1290	93	
	9	146	8	1210	121	
	13	219	15	1530	218	
	14	178	15	1260	87	
	15	165	13	943	124	120
	16	252	18	1690	191	to
	17	198	9	1210	104	200
	18	203	17	1020	77	from
	19	204	17	1205	205	each
	20	213	16	1050	72	plant
	64	304	36	1970	163	
	67	220	17	1210	171	
	68	219	9	1405	136	
	69	280	11	1820	75	
	70	219	14	1585	169	
	71	323	22	2060	280	
	72	262	14	1680	139	
	73	345	21	2105	160	

Standard errors are given in the table for the calculated values of cell number for each plant. It is obvious that real differences in cell number exist in both cortex and pith. Also it appears that cell number in the cortex is related to cell number in the pith. The value of the correlation is .653 ± .125. Further, within the F_2, cell number in the pith was related to the diameter of the stem at a distance of ten centimeters from the meristem. The value of the correlation was found to be .587 ± .182, in-

dicating that stem size and cell number are related. This relation also holds in the parents. Although in this F_2 the relation may be due to genetic linkage, it seems more plausible to assume that it is a structural necessity, and that the larger stems are larger through the possession of more cells than the smaller. This is of particular interest since it indicates that genetic differences may depend directly upon cell number.

DISCUSSION

Sinnott (1936) studied the relations between the primary tissues in a series of young shoots from a tree of *Pinus Strobus*, in a group of flower stalks of *Datura Stramonium*, and in a series of steles of the fern *Todea*

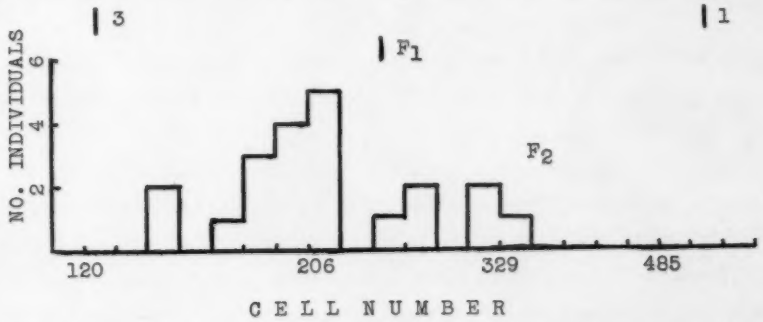


Fig. 10. Diagram shows the distribution of pith cell number in the F_2 of the cross between Bonnie Best and Red Currant. The positions of the mean values of the parents are indicated by vertical lines.

hymenophylloides. Considerable differences in stem size existed within these groups. He found in all of these that the diameter of the pith increased very much faster than the diameter of the stem, the diameter of the vascular cylinder slightly faster or at the same rate as the stem, and the diameter of the cortex very much more slowly than the diameter of the stem. He suggested several possible explanations for the position of the vascular cylinder; first, that it was determined by the position of the leaf primordia; then, that the vascular cylinder may be differentiated at the place where the two different growth rates of pith and cortex come together in the meristem; and lastly, that it is a matter of genetically determined pattern. He says (page 421), "No simple physiological explanation presents itself for these changes in relative size with increasing absolute size. This may be an expression of a developmental pattern inherent in the constitution of the organism."

Buchholz (1938) examined the relationships of tissue size in stems of different dimensions collected from branches of old trees of *Sequoia gigantea*. He found that diameter of stele, diameter of wood cylinder, and diameter of pith plotted against diameter of stem gave a value for "k" of 1.7. Thus pith and vascular tissue were growing at the same rate and very much faster than the stem. Although no figures are given for the cortical tissue it must have grown much more slowly than the stem. He also examined stems which bore male and female cones. The diameter of the stems bearing female cones was very much greater than those bearing male cones. In both, the cone-bearing branches had a relatively smaller pith and larger vascular cylinder than comparable vegetative stems. He found that large stem tips have broader promeristem points with many more cells than those of small stem tips. He presents a very interesting diagram of the promeristems from which the stems of the various types and sizes must have originated. He places all size differences as having their origin in the promeristem.

The two papers just cited both deal with stems which differ in size but not in age. This present paper, on the other hand, deals with size differences which at different levels within the individual plant are correlated with differences in age. Individual plants within a "pure" (i.e., inbred) line are so nearly identical that parts of one may be substituted for exactly corresponding parts of another. The differences among the "pure" lines themselves (the inbred varieties) and among the individual plants of the F_2 are, of course, genetic in origin. That it is possible to prove striking similiarities has been shown in the discussion of cell size in Bonnie Best and Red Currant, whereas extremely different cell and tissue relations were exemplified by the contrast between the two aforementioned varieties and Dwarf Champion.

The developmental study gives results which are not entirely in accordance with those of Sinnott. Thus, the gradient of growth rates found by him does not hold since in all the tomato varieties the vascular tissue grows most rapidly. However, in the latter some secondary growth occurred, although the curve which shows the relation of growth of vascular cylinder to stem tissue remains a straight line, indicating no change in relative growth within the beginning of secondary growth. Aside from this, the difference between rate of growth in cortex and pith holds as in Sinnott's material, although the differences are not as extreme as those found by him.

Within the primary tissues cell number seems to be determined in the meristem. Cell size is directly related to the distance of those cells from the meristem. The fact that the cells in the cortex are much smaller than those in the pith may be related to the slower growth of that tissue. The persistence of cell division in the epidermis may be related to the smaller ultimate size of the cells which may be under functional control. It is generally assumed that pith and cortical cells play a comparatively small rôle in the physiology of the plant. The evidence found here for cell number in general points directly to the conclusion that cell number may be genetically controlled without any influence upon cell size.

Between the "pure" lines (varieties) the differences are largely meristematic in origin. Cell number certainly is, and likewise size of pith. The rate of growth of vascular tissue is different in the different types but there is a regularity in the difference. The smaller the stem the more rapid the growth of vascular tissue. The reason for this may be connected with the physiology of the plant. Although the leaves are smaller on the small-stemmed types, the length of the internodes is the same, with one exception, so that approximately the same number of leaves is produced. Thus transpiration in the thin-stemmed types would not be a great deal less than in the thick-stemmed types. Although the vascular cylinder grows more rapidly there is never actually as much in the small-stemmed as in the large-stemmed types for the number of leaves which it supports. Numerous instances of increase in vascular tissue in response to demand exist in the literature. Of course, in this present case the differences in growth rates of the vascular tissue may be the result of genetic factors, but it seems more plausible to consider it in the nature of a secondary and physiological response.

The reason for the decreasing rate of growth in the cortex is not obvious. Since the growth rate of the cortex is smallest where the growth rate of the vascular tissue is greatest there appears to be a compensatory effect. This suggests that perhaps the functional demand cited in the previous paragraph may determine the distribution of food or growth hormones and so account for these differences in growth rate. This seems to be in contradiction to Sinnott's hypothesis that differences in rate of growth of cortex and pith determine the position of the vascular cylinder. It is difficult to imagine a mechanism of control which would act first on the cortex and secondarily on the vascular tissue since their relative functional importance is reversed. However, if the control is directly genetic such action is possible.

SUMMARY

The development of the stem was studied in three varieties of *Lycopersicum esculentum* and one variety of *L. pimpinellifolium*, in two hybrids between the species, and in one F_2 grown from one of the hybrids.

It was found that the rate of increase in diameter of stem with reference to the distance from the stem tip is the same in all types in spite of large initial and ultimate differences.

The length of internodes at corresponding distances from the meristem in plants of the same age is approximately the same in all types studied except in one variety of *L. esculentum*, Dwarf Champion, in which internode length is less. In general the internode below a branch is shorter than the one above. This branch effect is greatest in the thick-stemmed types.

The rate of growth of the different internal tissues is not the same. In general the pith increases in cross-sectional area at the same rate as the stem; the vascular cylinder enlarges faster and the cortex slower.

In the thinner-stemmed types the rate of growth of the vascular tissue as compared with growth of the stem is more rapid than in the thicker-stemmed types, and the rate of growth of the cortical tissue is correspondingly slower.

Cell cross-sectional area in the pith and in the cortex increases at the same rate as cross-sectional area of pith and cortex.

Epidermal cell number and cell size increase as the area of the stem increases.

Cell size in the cortex and in the pith in all but Dwarf Champion is the same at equal distances from the stem tip.

The differences in size of cortical and pith tissues in the different varieties is the result of differences in cell number. The hybrids lie intermediate between their parents and the positions of the F_2 plants indicate segregation and thus that cell number is inherited. No F_2 plant lies outside the cell number range of the parents.

The anatomical pattern possesses a regularity with relatively slight deviations which suggest a generic rather than a specific mechanism of control. Whereas minor deviations are under control of genetic factorial combinations that are characteristic of the several varieties and species, for *L. pimpinellifolium* has been held to be different specifically from *L. esculentum*, it is evident that for the genus *Lycopersicum* the mechanism involved is the same in all types.

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RUSTON, LOUISIANA

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Additions to Florida Fungi—II [1]

William A. Murrill

Russula Arnoldae sp. nov.

Pileo convexo-depresso, 8–9 cm. lato, atropurpureo, sapore grato; sporis globosis, 6–8μ, stipite roseo, 7 $\times$ 1.5 cm.

Pileus convex to slightly depressed, solitary, 8–9 cm. broad; surface slightly viscid, pruinose to glabrous, smooth, atropurpureous, darker at the center; margin even, entire, not peeling readily; context thick, white, unchanging, odorless, mild; lamellae adnate, some forked at the base, equal, broad, medium distant, entire, pallid to flavous; spores globose to subglobose, spinulose, ochroleucous under microscope, 1–guttulate, 6–8μ; stipe subequal, smooth, glabrous, roseous, 7 $\times$ 1.5 cm.

Type collected by Lillian E. Arnold on the ground in a high hammock at Sugarfoot, near Gainesville, Fla., Sept. 25, 1938 (*F 18230*). A very handsome, highly-colored species with yellow, spinulose spores and mild flesh.

Russula floridana sp. nov.

Pileo convexo-depresso, 8 cm. lato, viscido, glabro, purpureorubro; sporis albis, ellipsoideis, 6–8 $\times$ 4–6μ, stipite albo, 8 $\times$ 2 cm.

Pileus convex to somewhat depressed, solitary, 8 cm. broad; surface viscid, smooth, glabrous, uniformly purple-red, cuticle not readily separable, margin entire, tuberculate-striate; context white, mild, odorless, yellowish when dry; lamellae adnate, mostly equal, medium distant, broad, entire, white, gray in dried specimens; spores white in mass, broadly ellipsoid, distinctly echinulate, 1–guttulate, 6–8 $\times$ 4–6μ; stipe equal, smooth, glabrous, white, slightly grayish or yellowish when dry, 8 $\times$ 2 cm.

Type collected by W. A. Murrill on the ground in woods at Gainesville, Fla., June 30, 1938 (*F 18084*). A handsome species of rare occurrence.

Russula heterospora cremea var. nov.

Pileo cremeo, lamellis sinuatis, sporis ellipsoideis, glabris, 10 $\times$ 6μ, stipite albo, 4 $\times$ 1–1.7 cm.

Pileus convex to depressed, solitary, 7 cm. broad; surface slightly viscid, smooth, glabrous, cremeous with a few rusty spots, peeling very readily, margin even; context thin, white, mild; lamellae distinctly sinuate, broad, subdistant,

[1] Numbers cited in this article refer to specimens in the herbarium of the Florida Agricultural Experiment Station, at Gainesville.

some forked at the middle, white; spores cremeous in mass, ellipsoid, smooth, hyaline, about 10 × 6μ; stipe tapering downward, smooth, white, glabrous, 4 × 1–1.7 cm.

Type collected by West, Arnold and Murrill on the ground in a high hammock at Sugarfoot, near Gainesville, Fla., Sept. 29, 1938 (*F 18343*). Very different in appearance from the typical form of *R. heterospora* Beardslee so common about Gainesville, which is rosy-purple-olivaceous on the surface and has more slender spores.

Russula tuberculata sp. nov.

Pileo convexo-depresso, 6 cm. lato, praestriato, albo et rubro; lamellis angustatis, sporis globosis, tuberculatis, 8–11μ; stipite roseo-albo, 4 × 1.5–2 cm.

Pileus convex to depressed, solitary, 6 cm. broad; surface somewhat viscid, glabrous, conspicuously striate, not peeling readily, white with reddish patches or spots over the center; context thin, white, mild, odorless; lamellae adnate, equal, many forked at the base, medium distant, narrow, white, entire; spores perfectly globose, coarsely tuberculate, white in mass, 8–11μ; stipe flattened, tapering downward, smooth, glabrous, white tinged with rose, 4 × 1.5–2 cm.

Type collected by West, Arnold and Murrill on the ground in a high hammock at Sugarfoot, near Gainesville, Fla., Sept. 29, 1938 (*F 18344*). Very striate with narrow gills and globose spores covered with large papillate warts.

Lactaria parvula sp. nov.

Pileo convexo-depresso, 2 cm. lato, pallido, zonato, sapore grato, lacte albo; sporis subglobosis, 4–5μ, stipite albo, 3 × 0.6 cm.

Pileus convex to slightly depressed, solitary, 2 cm. broad; surface slightly viscid when moist, smooth, glabrous, zonate, whitish with a rosy-isabelline tint, margin entire, even; context white, unchanging, mild; lamellae tapering behind, arcuate, narrow, crowded, forked, entire, white, unchanging; latex white, unchanging; spores globose or subglobose, finely tuberculate, hyaline, 4–5μ; sterile cells abundant, conic, pointed, hyaline, projecting about 15 × 8μ; stipe equal, smooth, glabrous, white, unchanging, 3 × 0.6 cm.

Type collected by E. West and W. A. Murrill on the ground in Sanchez Hammock, eleven miles northwest of Gainesville, Fla., July 23, 1938 (*F 18214*). A small, whitish species with small spores and large, conic sterile cells on the edges of the gills.

Lactaria Westii sp. nov.

Pileo convexo-depresso, 5–7 cm. lato, pallido, piperato; sporis globosis, valde tuberculatis, 9μ; stipite albo, pruinoso, 2–4 $\times$ 0.8–1.3 cm.

Pileus convex to slightly depressed, solitary to gregarious or subcespitose, 5–7 cm. broad; surface soft, dry, minutely pruinose to glabrous, smooth, varying from white to pale avellaneous-isabelline, pallid or partly isabelline when dry, margin even, entire, glabrous; context white, unchanging, odorless, acrid; lamellae attenuate behind, slightly decurrent, narrow, close to medium distant, inserted, entire, yellowish to dirty-brownish-yellow; latex white, unchanging; spores perfectly globose, strongly tuberculate, hyaline, about 9μ; stipe usually short, equal or tapering downward, smooth, pruinose, especially above, white, unchanging, 2–4 $\times$ 0.8–1.3 cm.

Type collected by E. West and W. A. Murrill under an oak at Newnan's Lake, Fla., July 30, 1938 (*F 18218*). Also collected by West and Murrill under oaks at Kanapaha Sink, July 26, 1938 (*F 18085*), and at Arredonda, July 29, 1938 (*F 18086*). Suggesting *L. praeseriflua* Murrill when first seen but finely pruinose to glabrous and having acrid milk.

Entoloma Grayanum caespitosum var. nov.

Pileo convexo, caespitoso, sporis 10 $\times$ 8μ; stipite albo, 4–5 $\times$ 0.5–1 cm.

Pileus broadly convex, densely cespitose, 4–5 cm. broad; surface smooth, glabrous, opaque, avellaneous-umbrinous, margin incurved, even, entire to undulate; context white, taste and odor strongly farinaceous; lamellae adnate, pallid to pale-pink; spores decidedly angular, apiculate, 1–guttulate, pink, about 10 $\times$ 8μ; stipe smooth, white, glabrous, tapering upward, 4–5 $\times$ 0.5–1 cm.

Type collected by W. A. Murrill on the ground in woods at Gainesville, Fla., July 1, 1938 (*F 18248*). Densely clustered and having a shorter stipe than the typical form.

Entoloma pinicola sp. nov.

Pileo convexo-subexpanso, 2.5–3.5 cm. lato, albido, disco isabellino; sporis angulatis, 9–11 $\times$ 6–7μ, stipite albo, 5 $\times$ 0.3–0.5 cm.

Pileus convex to subexpanded, gregarious, 2.5–3.5 cm. broad; surface smooth, glabrous, satiny, whitish, isabelline on the conic umbo, margin even, entire; context very thin, odorless, mild to slightly astringent, white; lamellae sinuate with decurrent tooth, ventricose, inserted, broad behind, medium distant, the edges uneven and more or less toothed; spores decidedly angular, usually 5–sided or kite-shaped, apiculate, 1–2–guttulate, pale-pink, 9–11 $\times$ 6–7μ;

cystidia none; stipe tapering upward, smooth, glabrous, shining, white, about 5 × 0.3–0.5 cm.

Type collected by West, Arnold and Murrill on the remains of a much-decayed pine log in Prairie Creek Hammock, near Gainesville, Fla., July 15, 1938 (*F 18252*). Unusual in its color, substratum, and slender stem. Herbarium specimens are umbrinous throughout and show white streaks of some compound of calcium on the surface of the cap.

Entoloma subcommune sp. nov.

Pileo convexo-subdepresso, umbonato, 2.5–3 cm. lato, avellaneo-umbrino, odore et sapore farinaceo; sporis angulatis, 8–10 × 6–7μ, stipite avellaneo, 4 × 0.3 cm.

Pileus convex to slightly depressed with a small flat umbo, gregarious to cespitose, 2.5–3 cm. broad; surface smooth, glabrous, avellaneous-umbrinous, blackish when dry; margin even, entire or slightly lobed; context thin, white, with strong farinaceous odor and taste; lamellae sinuate, broad behind, fragile, white to pink, inserted, medium distant, edges thin, undulate to eroded; spores decidedly angular, apiculate, 1–2–guttulate, pink, broadly ellipsoid in outline, 8–10 × 6–7μ; cystidia none; stipe equal, smooth, glabrous, avellaneous, whitish-mycelioid at the base, about 4 × 0.3 cm.

Type collected by West and Murrill on the ground in Kelley's Hammock, ten miles northwest of Gainesville, Fla., Aug. 3, 1938 (*F 18241*). Also collected by Murrill, Weber and West near Gainesville, Dec. 14, 1926 (*F 10020*). Near *E. commune* Murrill but with different spores.

Nolanea alachuana sp. nov.

Pileo convexo-umbilicato, 1–2.5 cm. lato, striato, avellaneo; sporis angulatis, 7–9μ, stipite avellaneo, 3–5 × 0.1–0.3 cm.

Pileus conic to broadly convex, not fully expanding, usually umbilicate, gregarious or scattered, 1–2.5 cm. broad; surface avellaneous, glabrous, shining, striate-sulcate to the disk, margin entire; context thin, pallid, odorless, mild; lamellae sinuate, broad, triangular, medium distant, inserted, entire, pallid to dull-pink, at length dark-isabelline; spores globose in outline, moderately angular, strongly apiculate, 1–guttulate, 7–9μ; cystidia none; stipe equal, smooth, glabrous, shining, concolorous, whitish-mycelioid at the base, 3–5 × 0.1–0.3 cm.

Type collected by West and Murrill on the ground in Kelley's Hammock, ten miles northwest of Gainesville, Fla., July 19, 1938 (*F 18246*). Also at the same place, July 19, 1938 (*F 18243*), and Aug. 3, 1938

(*F 18242*). The spores distinguish it at once from species of similar appearance. They remind one of grains of corn.

Cortinarius Arnoldae sp. nov.

Pileo convexo, praeviscido, badio, 4 cm. lato; lamellis lilacinis, sporis tuberculatis, 15–17 × 6–7μ; stipite ventricoso, lilacino, 9 × 1–1.5 cm., annulo fibroso, albo.

Hymenophore covered with a thick, slimy universal veil resembling the white of an egg; pileus campanulate, not fully expanded, slightly umbilicate, solitary, 4 cm. broad; surface smooth, glabrous, uniformly badious, margin even, entire, incurved, lilac; context thin, pallid, mawkish, odorless; lamellae adnate, arcuate, subdistant, inserted, entire, pale-lilac, becoming fulvous; spores pip-shaped from above, turtle-shaped from the side, coarsely tuberculate, deep-ferruginous, 15–17 × 6–7μ; stipe oblong-fusiform, smooth, glabrous, lilac, dirty-cremeous at the base, 9 × 1–1.5 cm.; annulus ample, white, cobwebby, attached 1 cm. from the apex of the stipe.

Type collected by Lillian E. Arnold on the ground in a high hammock at Sugarfoot, near Gainesville, Fla., Sept. 29, 1938 (*F 18352*). A beautiful species, very rare, with spores shaped like a young box-turtle and covered with warts like a hop-toad. I have never seen a more glutinous agaric. *C. splendidus* Peck is a near relative.

Ceriomyces rubricitrinus sp. nov.

Pileo convexo, 5–8 cm. lato, glabro, testaceo ad latericio; tubulis citrinis, sporis fusoideis, 15 × 5μ; stipite reticulato, rubropunctato, 5 × 1.5–1.8 cm.

Pileus broadly convex, gregarious, 5–8 cm. broad; surface smooth, glabrous, testaceous to latericious, margin even, entire; context white with a yellowish tint, quickly turning blue when wounded, taste subacid; tubes slightly depressed at the stipe, slender, citrinous, becoming blue when wounded, mouths small, not stuffed; spores oblong-fusiform, smooth, yellowish-brown, 15 × 5μ; stipe tapering upward, 5 × 1.5–1.8 cm., yellow above, reddish-dotted below, reticulate, roseous within when cut except at the base, where it becomes bluish.

Type collected by W. A. Murrill on a lawn near a laurel oak in Gainesville, Fla., July 2, 1938 (*F 17321*). I first tried to make this a red variety of *C. subsensibilis* Murrill but the differences were too great. Only the one collection was made.

Gyroporus deflexus sp. nov.

Pileo convexo, 4.5 cm. lato, atro-griseo, subtomentoso, sapore grato; tubulis albis, parvis, sporis ochroleucis, 10–12 × 3μ; stipite glabro, 5 × 1 cm.

Pileus convex, gregarious, 4.5 cm. broad; surface dry, smooth, subtomentose, dark-avellaneous to umbrinous, margin entire, sterile, deflexed; context thick, white, unchanging, sweet; tubes plane, small, white, becoming yellowish where bruised; spores oblong-fusiform, smooth, ochroleucous, 10–12 × 3μ; stipe equal, glabrous, white above, avellaneous below, marked with parallel ridges, 5 × 1 cm.

Type collected by West, Arnold and Murrill on the ground in rather dry oak-pine woods at Magnesia Springs, Fla., July 15, 1938 (*F 18216*). Similar to *Ceriomyces subtomentosus* (L.) Murrill in shape but dark-gray in color with small, white tubes and strongly deflexed margin.

Gyroporus Rhoadsiae sp. nov.

Pileo convexo-subexpanso, 6–8 cm. lato, albido, felleo; sporis fusoideis, hyalinis, 12 × 4μ; stipite albo, reticulato, 6–8 × 1.5–2 cm.

Pileus convex to subexpanded, thick, gregarious, 6–8 cm. broad; surface dry, smooth, glabrous, whitish, shining when dried; margin thin, even, entire, projecting; context moderately thick, white, unchanging, very bitter; tubes angular, 5–10 mm. long, 1–2 to a mm., thin-walled, white, unchanging; spores oblong-fusiform, smooth, hyaline, about 12 × 4μ; stipe equal or nearly so, glabrous, beautifully reticulate above, white, unchanging, 6–8 × 1.5–2 cm.

Type collected by Louise and Arthur S. Rhoads on the ground under slash pine and shrubs at the margin of Lake Rosa, two miles east of Melrose, Fla., Sept. 8, 1938 (*F 18199*). A large, whitish, bitter species, becoming shining and almost pure-white when dried.

Gyroporus stramineus sp. nov.

Pileo convexo-subexpanso, 6 cm. lato, stramineo, sapore grato; sporis ochroleucis, 9–14 × 3μ, stipite albo, 4 × 1 cm.

Pileus convex subexpanded, solitary, 6 cm. broad; surface dry, smooth, glabrous, stramineous, whitish and shining when dried; margin even, undulate, fertile; context white, mild; tubes somewhat decurrent, 5 mm. long, 2–3 to a mm., isabelline at maturity, fulvous when dried; spores sausage-shaped, smooth, ochroleucous, 9–14 × 3μ; stipe equal, smooth, glabrous, white, unchanging, about 4 × 1 cm.

Type collected by W. A. Murrill in turkey-oak woods at Gainesville, Fla., Sept. 10, 1938 (*F 18156*). There are no rosy tints as in *B. roseialbus* Murrill; the flesh is mild; and the stem not at all reticulate.

Boletus pseudogranulatus sp. nov.

Pileo convexo-plano, 6 cm. lato, viscido, roseo-isabellino, subfelleo; tubulis ochroleucis, non punctatis, sporis fusoideis, 8–10 × 2.5–3μ; stipite sulphureo, reticulato, 4 × 1.5–2 cm.

Pileus convex to plane, gregarious, about 6 cm. broad; surface decidedly viscid, dark rosy-isabelline, margin even, projecting, appendiculate; context white, unchanging, slightly bitter and mawkish, reaching 1 cm. thick; tubes adnate or decurrent, reaching 5 mm. long, 2–4 to a mm., not stuffed, ochroleucous, not changing when cut; edges entire, without resinous dots; spores oblong-fusiform, smooth, granular, hyaline under the microscope, 8–10 × 2.5–3μ; stipe enlarged below, solid, reticulate nearly to the base, sulphur-yellow without and within, without resinous dots, 4 × 1.5–2 cm.; veil so reduced that only a few fibers are left on the stipe.

Type collected by W. A. Murrill on the ground under a pine in Gainesville, Fla., Oct. 2, 1938 (*F 18342*). It was surprising to find specimens so similar to *R. granulatus* (L.) P. Karst. without resinous dots on either tubes or stem and having the latter beautifully reticulated. In drying the context first changes to yellow and at length to rosy-isabelline, while the tubes become melleous.

Since this species has no true annulus it does not rightfully belong in the genus *Boletus* as limited by me, but there is no other place to put it. In *Rostkovites* glandular dots are essential and my conscience will not allow me to assign it to *Gyroporus*, so far removed from its obvious relatives. In *Boletus* there are species like *B. Clintonianus* Peck without glandular dots and with slight reticulations on the stipe; so let us consider our present problem child as having a very much reduced veil, which leaves only fragments on the margin and a ring-trace on the stipe.

Tyromyces leucomallellus sp. nov.

Pileo effuso-reflexo, 0.5–2 cm. lato, albo; tubulis 1–2 mm. longis, angulatis, albis; sporis 4–5 × 1μ.

Effused-reflexed, mostly resupinate, subcircular to elliptic, 0.5–2 cm. broad, the reflexed portion an elevated margin or a narrow strip a few millimeters wide; surface soft, pubescent, azonate, white, becoming slightly ferruginous when bruised; margin thin, even, undulate; context thin, fibrous, white, unchanging; tubes 1–2 mm. long, 3–4 to a mm., angular, thin-walled, entire, white, brownish where bruised, collapsing and somewhat lacerate-dentate with age; spores copious, allantoid, smooth, hyaline, 2–3-guttulate, about 4–5 × 1μ.

Type collected by W. A. Murrill on a dead log of *Taxodium distichum* at Newnan's Lake, Fla., April 24, 1938 (*F 18231*). Cotype the same time,

place and host (*F 18239*). An inconspicuous white species somewhat resembling *T. leucomallus* (Berk. & Curt.) Murrill and in some ways suggesting *Spongipellis fragilis* (Fr.) Murrill, but much smaller than either and almost wholly resupinate. The guttae in the spore are very large, appearing like a row of beads.

Tyromyces magnisporus sp. nov.

Pileo turbinato, 4–5 × 2–3 cm., subtomentoso, albo vel cremeo, praefelleo; tubulis albis, angulatis, sporis ovoideis, 10–12 × 5–7μ.

Pileus irregularly turbinate, usually clasping, subimbricate, solitary, 4–5 cm. broad and 2–3 cm. thick behind; surface plane or uneven, finely tomentose, white or cremeous, margin even, very irregular, undulate or lobed, fertile; context very bitter, fragant like anise, spongy-fleshy, fragile when dry, white, unchanging; tubes about 5 mm. long, 2–3 to a mm., rather thin-walled, angular, entire, white, usually becoming isabelline on drying; spores elongate-ovoid or subellipsoid tapering at one end, smooth, hyaline, often 1–guttulate, copious, 10–12 × 5–7μ.

Type collected by West and Murrill on a small dead hardwood stub in Kelley's Hammock, ten miles northwest of Gainesville, Fla., July 19, 1938 (*F 17916*). Also collected by West and Murrill on a dead hardwood stub in Sanchez Hammock, July 23, 1938 (*F 18238*), and on a dead hardwood stub in Kelley's Hammock, Aug. 3, 1938 (*F 17395*). Conspicuous by reason of its encircling habit, large spores, and bitter taste.

Tyromyces Newellianus sp. nov.

Pileo effuso-reflexo, imbricato, azonato, ochraceo, rugoso; tubulis, albis, parvis, sporis 3–4 × 1μ.

Pileus effused-reflexed, imbricate, the reflexed portion sessile, dimidiate to laterally elongate, 1–1.5 × 1–4 × 0.2–0.4 cm.; surface azonate, rough, scabrous to rugose or subvirgate, ochraceous, isabelline when dried; margin thin, even, glabrous, entire to undulate; context thin, white, unchanging, friable; tubes slender, angular, thin-walled, 3–4 to a mm., white and unchanging within, edges entire to dentate, white, becoming ferruginous when bruised and somewhat collapsed on drying; spores allantoid, smooth, hyaline, copious, about 3–4 × 1μ; cystidia none.

Type collected by W. A. Murrill on a rotten hardwood log in a hammock at Gainesville, Fla., Sept. 23, 1938 (*F 18215*). Having the type of spore found in *T. caesius* (Schrad.) Murrill but with color and surface entirely different. Dedicated to Dr. Wilmon Newell, for many years Director of the Florida Agricultural Experiment Station.

Tyromyces pini-glabrae sp. nov.

Pileo semi-resupinato, 1.5–3 cm. lato, tomentosulo, albo; tubulis merulioideis, albis, sporis globosis, 3–4μ.

Pileus semi-resupinate, 1.5–3 cm. broad, the reflexed portion dimidiate to elongate, thin, flexible, becoming very rigid on drying, 0.5–1 $\times$ 1–3 $\times$ 0.1–0.2 cm.; surface uneven, slightly tomentose, milk-white, unchanging; margin even, undulate to lobed, slightly opaque-brownish where bruised, decurved on drying; context homogeneous, hygrophanous, whitish, appearing horny and brownish when dry; hymenium merulioid when young, mature tubes shallow, irregular, angular, thin-walled, 2–4 to a mm., white, unchanging, entire; spores few, globose, smooth, hyaline, 3–4μ.

Type collected by West, Arnold and Murrill on a dead log of *Pinus glabra* Walt. in Beech Woods, near Santa Fé, Fla., July 13, 1938 (*F 18235*). A milk-white species with very shallow pores found once in the only beech grove known in Alachua County.

Tyromyces pseudolacteus sp. nov.

Pileo dimidiato, 2–3 cm. lato, hirsuto, albo; tubulis parvis, albis, brevibus sporis ovoideis, 3 $\times$ 2μ.

Pileus sessile, imbricate, dimidiate to reniform, convex, 1–2 $\times$ 2–3 $\times$ 0.5–1 cm.; surface sodden, azonate, rough, hirsute or hirsute-tomentose, white, unchanging; margin acute, even, entire to undulate, white, becoming brown when dried; context homogeneous, soft, fibrous, white, unchanging, drying soft-corky and subfragile, reaching 1 cm. thick; tubes delicate, short, angular, thin-walled, 5–6 to a mm., white, unchanging, entire to dentate; spores ovoid, smooth, hyaline, 1-guttulate, about 3 $\times$ 2μ; cystidia none.

Type collected by West and Murrill on a fallen dead oak stick in woods at Gainesville, Fla., June 3, 1938 (*F 17396*). Suggesting the true *Polyporus lacteus* of Fries as it occurs in Sweden but without the allantoid spores of that species. An older specimen measuring 4 $\times$ 5 $\times$ 2 cm., which seems to belong here, has the same hirsute surface and similar spores but the slender tubes have partially collapsed and the edges are lacerate. It was collected by West and Murrill on an oak log in Gainesville, Oct. 24, 1932 (*F 18253*).

Poria cubitispora sp. nov.

Prae-effuso, adnato; tubulis albis, 3–4 mm. longis, parvis, angulatis, praefelleis, fragilibus; sporis allantoideis, hyalinis, 2.5–3 $\times$ 1μ; cystidiis 30 $\times$ 10μ.

Widely effused, continuous for many centimeters, inseparable, about 5 mm. thick; margin slight, appressed, white, soon inconspicuous; context very thin,

white; hymenium even, glistening, white with a few luteous stains, dark-isabelline where bruised, cremeous when dried; tubes 3–5 mm. long, about 5 to a mm., soft, juicy, fragile, intensely bitter, mouths fairly regular, angular, edges thin, entire; spores short, sharply curved, smooth, hyaline, very abundant, 2.5 × 1μ; cystidia abundant, tapering from a thick base, pointed or obtuse, encrusted, projecting about 30 × 10μ.

Type collected by West, Arnold and Murrill on the side of a much-decayed log of *Nyssa sylvatica* Marsh. in a high hammock at Sugarfoot, near Gainesville, Fla., Sept. 29, 1938 (*F 18341*). A striking species as bitter as quinine, with spores that remind one of boiled shrimp. In *P. griseoalba* (Peck) Sacc. and *P. sulphurella* (Peck) Sacc. the spores measure about 4 × 1μ and vary from cylindric to slightly allantoid. In this species they are short and curved almost as much as an elbow joint. Under the microscope they seem to wriggle about like half-coiled insect larvae. Mr. West assisted me with the microscopic examination.

NEW COMBINATIONS

For those using Saccardo's nomenclature the following new combinations are made:

Ceriomyces rubricitrinus = **Boletus rubricitrinus**
Gyroporus deflexus = **Boletus deflexus**
Gyroporus Rhoadsiae = **Boletus Rhoadsiae**
Gyroporus stramineus = **Boletus stramineus**
Tyromyces leucomallellus = **Polyporus leucomallellus**
Tyromyces magnisporus = **Polyporus magnisporus**
Tyromyces Newellianus = **Polyporus Newellianus**
Tyromyces pini-glabrae = **Polyporus pini-glabrae**
Tyromyces pseudolacteus = **Polyporus pseudolacteus**

HERBARIUM FLORIDA AGRICULTURAL EXPERIMENT STATION
GAINESVILLE, FLORIDA

Descriptions of Tropical Rusts—II [1]

GEORGE B. CUMMINS

(WITH TEN FIGURES)

Puccinia amphiospora (Jacks. and Holw.) comb. nov. (fig. 1). (*Uredo amphiospora* Jacks. and Holw.; Jackson in Mycologia **24**: 72. 1932.)

Telia not seen; teliospores in the uredia ellipsoid or oblong-ellipsoid, rounded above, usually narrowed below, moderately constricted at the septum, 16–20 × 29–38μ; wall light cinnamon-brown, smooth, 1μ thick, the pore in the upper cell apical and covered by a small, hyaline umbo which disappears at germination, pore in lower cell at septum; pedicel hyaline, fragile, about one-half the length of the spore but usually broken near the spore. The spores germinate at once.

Jackson (*l.c.*) reported three collections on *Hyptis spicata* Poit. from Bolivia, two of which were issued in Reliq. Holw. as numbers 415 and 420. Both of them have teliospores. The type was collected by the Holways at Cochabamba, Bolivia, Feb. 25, 1920 as number 324 (Reliq. Holw. 415).

The occurrence of two kinds of urediospores, one of which is considered to be amphisporic, is the characteristic feature of the species.

Puccinia unilateralis (Arth.) comb. nov. (fig. 8). (*Uredo unilateralis* Arth., Bull. Torrey Club **45**: 155. 1918.)

Pycnia epiphyllous, 2 or 3 in a group, subepidermal, globoid, 130–200μ diam. Aecia hypophyllous opposite the pycnia, few in a group on spots up to 3 mm. diam., subepidermal, caeomoid, blister-like, orange, usually elongate, 0.1–0.3 × 0.5–1 m. or larger and irregular by confluence, peridium none; aeciospores catenulate, oblong, broadly ellipsoid or globoid, 20–28 × 26–35μ; wall 2μ thick, hyaline, closely and rather coarsely verrucose with squarish or irregular, flat tubercles. Uredia hypophyllous, scattered or in more or less circinate groups, 0.2–0.8 mm. diam., pulverulent, dark cinnamon-brown; urediospores asymmetrical, obovoid with the pore face-view, reniform with the pore lateral, 20–26 × 26–32μ; wall cinnamon-brown, 1.5μ thick, echinulate except around the single pore located somewhat below the equator in the concave surface. Telia hypophyllous, subepidermal, pulvinate, yellowish, waxy in appearance but becoming cinereous from germination; teliospores sessile on a cellular hymenium, oblong or clavate, rounded above, narrowed below, constricted at the septum, 18–27 × 45–70μ; wall hyaline, 1–1.5μ thick at sides,

[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

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6–12μ at apex, less so or similarly thickened below the septum; the pore apical in upper cell, next the septum in lower cell. The teliospores germinate at once.

The description of pycnia and aecia was drawn from a specimen collected by J. H. Faull, Mt. San Felipe, Oaxaca, Mexico, Dec. 5, 1938 on *Geranium* sp. Kern and Whetzel (Journ. Dept. Agric. Porto Rico **14**: 347.

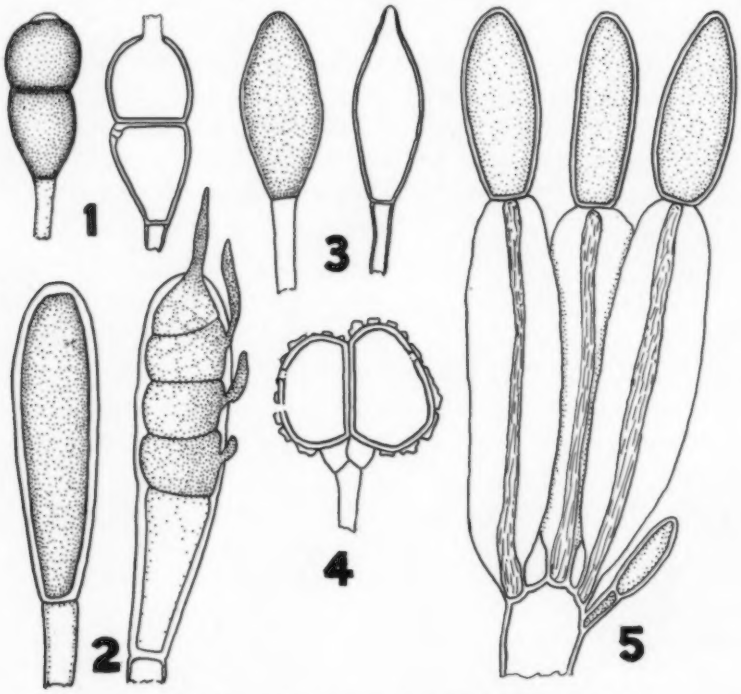

Fig. 1. Teliospores of *Puccinia amphiospora*. ×650.
Fig. 2. Teliospores of *Acrotelium lucumae*. ×650.
Fig. 3. Teliospores of *Scopella cryptostegiae*. ×650.
Fig. 4. One teliospore of *Dicheirinia solenioides*. ×650.
Fig. 5. Teliospores of *Scopella bauhiniicola*. ×650.

1930) have previously described pycnia and aecia of what probably should be considered the same species on *G. hirtum* Willd. from Colombia (*Chardon 596*).

Telia are present in Faull's specimen but are described from the type of *Uredo unilateralis* on *G. mexicanum* (issued in Barth. N. Am. Ured. *2481*) where they were found to be numerous. Telia are also present on Chardon's specimen, which Dr. F. D. Kern kindly sent me. The teliospores differ noticeably from North American specimens in being narrower (14–18 × 58–73μ) and in having a thinner apical wall (2–4μ). No differ-

ences were evident in the aecia and uredia. The species is known to occur also in Ecuador on *G. chilloense* Willd. and *G. sodiroanum* Kunth but the collections have only uredia.

The attachment of the teliospores is difficult to observe in either crushed mounts or sections. No pedicels have been seen. The spores appear rather to be sessile upon a basal cellular hymenium with two or three attached to the unit cells which are neither elongate nor laterally free.

Puccinia poikilospora sp. nov. (fig. 10).

Pycnia et aecia ignota. Uredia hypophylla, subepidermalia, pustulata, sparsa vel aggregata, cinnamoneo-brunnea; urediosporae obovoideae, ellipsoideae vel late ellipsoideae, 18–23 × 25–33μ; membrana pallide cinnamomeo- vel aureo-brunnea, 2–3μ cr., moderate echinulata, poris germ. 3 vel 4 aequatorialibus. Telia hypophylla, subepidermalia, pustulata, sparsa vel circinata, brunneo-atra; teliosporae variabiles, ex cellulis 2–4 compositis, oblongae vel clavatae, 16–23 × 33–50μ; membrana 1.5–2μ cr., 4–9μ supra, castaneo-brunnea; pedicellis aureo-brunneis, 20–45μ longis.

On *Smilax spinosa* Mill., Jutiapa, Guatemala, Dec. 21, 1938, *J. R. Johnston 1425*. Type specimen in the Arthur Herbarium, Purdue University Agricultural Experiment Station.

This interesting rust has much the gross appearance of *Puccinia smilacis* Schw. but the sori are larger, more blister-like and the epidermis usually opens by a slit. The telia have a tendency to occur in a circle about the uredia and, like the uredia, remain partially covered by the epidermis. Variation in the septation of the teliospores is conspicuous, with two-, three- and four-celled spores occurring in approximately equal proportions. Mesospores are rare. The cells may all be arranged longitudinally or the upper two may be horizontally arranged upon the subjacent cell. Still greater irregularity in conformation occasionally occurs.

Bubakia erythroxylonis (Graz.) comb. nov. (*Uredo erythroxylonis* Graz., Bull. Soc. Myc. Fr. **7**: 152. 1891.)

Telia hypophyllous, circinate about the uredia, subepidermal, blackish brown, 35–100μ in diameter or becoming confluent; teliospores in crusts 2–4 spores thick, individual spores irregularly arranged, not catenulate, oblong or cuboid, 7–12 × 12–26μ, the lower ones usually irregular; wall chestnut-brown, smooth, 1μ thick at sides, 1.5–2.5μ apically in the outer spores, not strongly adherent laterally, sessile.

This description of telia is based upon a collection on *Erythroxylon havanense* Jacq., Vivijagua, Isle of Pines, Cuba, Feb. 28–29, 1916, *N. L. Britton, E. G. Britton* and *Percy Wilson 15023*.

The telia are developed by the branching and septation of a few hyphae and usually originate beneath stomata. Prior to the formation of spores the hyphae become thick-walled and produce a cellular complex. The unit cells of this complex enlarge, become pigmented and ultimately become spores. The uppermost layer of spores is regularly arranged beneath the epidermis but the lower ones lack this regularity and vary more in shape. This irregularity within the sorus becomes more noticeable the larger the sorus becomes.

Dicheirinia solenioides (P. Henn.) comb. nov. (fig. 4). (*Uredo solenioides* P. Henn., Hedwigia **35**: 250. 1886.)

Telia not seen; teliospores in the uredia 2–celled, broadly ellipsoid, broader than high, vertically septate, constricted at the septum, 30–38μ broad by 23–26μ high; wall 1.5μ thick, chestnut-brown or darker, coarsely tuberculate with cubical or irregular warts 2–3μ high, pores one in each cell above the equator in the outer wall; pedicel short and fragile, hyaline, thin-walled, simple basally but horizontally 2–celled next the spore.

Hennings (*l.c.*) in his original description mentions having seen one teliospore and the Sydows (Monogr. Ured. **4**: 490. 1924) report that they also observed teliospores but were not convinced that they belonged with the uredia. There is no doubt that they do, however, because I have seen them attached in sections of the uredia.

This characteristic and appropriately named species is similar in appearance to *D. ormosiae* (Arth.) Cumm. The sori are abundantly provided with peripheral paraphyses which extend well above the surface of the host, curve inward and produce a sorus "solenioid" in appearance. *D. ormosiae* also has such a sorus but with the paraphyses much branched and botryoid rather than simple. Both species have a single pore in the urediospore, located next the hilum.

These studies are based upon three specimens, all collected by Ule at St. Catharina, Sao Francisco, Brazil on *Nectandra rigida* Nees. One bears Ule's number 90 but is without date, the second was issued in Rabenh. Fungi eur. *4243* and the third was collected April, 1885 but is without a number. Perhaps all represent a single collection.

Acrotelium lucumae (Arth. and Johnst.) comb. nov. (figs. 2, 7). (*Uredo lucumae* Arth. and Johnst., Mem. Torrey Club **17**: 169. 1918.) (*Uraecium lucumae* Arth., Bull. Torrey Club **60**: 467. 1933.)

Telia hypophyllous, developing in the uredia or separately, subepidermal, round, oval or irregular in outline, 0.1–1.0 mm. in diameter, waxy, yellowish;

teliospores 1–celled, borne in groups on light brownish or hyaline, laterally free basal cells, pedicellate, cylindrical, rounded above, obtuse below, contents yellowish, 15–20 × 59–98μ in mature condition; wall hyaline, apparently slightly hygroscopic, smooth, 2.5–3μ thick; pedicel hyaline, thin-walled, about one-half as long as the spore and nearly as broad. The teliospores germinate at once by the formation of a 4–celled internal basidium in the upper one-half of the spore.

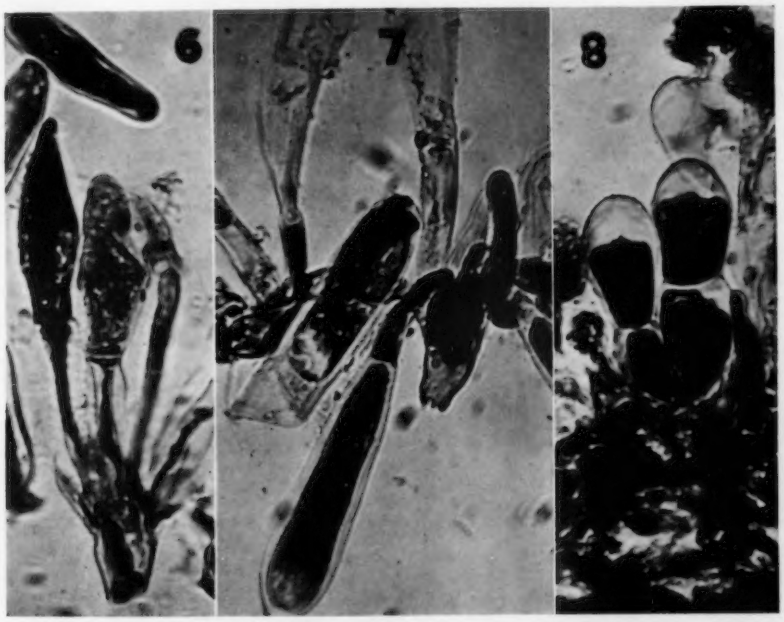

Fig. 6. *Scopella bauhiniicola;* photograph of a basal cell and attached teliospores (stained). ×625.

Fig. 7. *Acrotelium lucumae;* photograph of a basal cell and attached teliospores (stained). ×500.

Fig. 8. *Puccinia unilateralis;* photograph of a free-hand section of a telium, showing two teliospores (stained). ×650.

The description is drawn from a specimen received from Dr. Erdman West of the Florida Agricultural Experiment Station, collected by Geo. Ruehle at Homestead, Dade Co., Florida, Feb. 7, 1939. Telia are also present on the following specimens in the Arthur Herbarium: Santiago de las Vegas, Cuba, Feb. 23, 1916 and Mar. 5, 1916, J. R. Johnston *490* and *494*. All are on *Lucuma nervosa* A.DC.

The only previously described species of this genus is *Acrotelium ichnocarpi* Syd., in the Philippine Islands. Both *A. ichnocarpi* and *A. lucumae* have cylindrical, thick-walled teliospores but the basal cells

are much more conspicuous and larger in *A. lucumae*. Basal cells are not readily observable in *A. ichnocarpi*.

In *A. lucumae* the pycnia are subcuticular and the aecia uredinoid and similar to the uredia which follow. Only uredia and telia have been reported for *A. ichnocarpi*. There is, therefore, no very broad basis upon which to judge the relationship of the two species.

Scopella cryptostegiae (Vestergr.) comb. nov. (fig. 3). (*Uredo cryptostegiae* Vestergr., Svensk. Bot. Tidskr. **8**: 90. 1914).

Telia following in the uredia or separate, subepidermal, waxy in appearance, yellowish, pulvinate, small 0.1–0.2 mm. in diameter; teliospores produced in groups on straight or apically curved, laterally free basal cells, ellipsoid or ovoid, contents yellowish, 12–17 × 27–38μ; wall hyaline, smooth, uniformly 0.5–1.0μ thick, without a germ pore; pedicel shorter than or equal to the length of the spore, hyaline, thin-walled. The teliospores germinate at once by the apical elongation of the spore.

This description is drawn from the type collection on *Cryptostegia madagascariensis* Boj., collected by B. Palm at Majunga, Madagascar, April, 1912 and issued in Vestergr. Microm. rar. sel. *1660*.

The Sydows (Monogr. Ured. **4**:431. 1924) considered that the species belonged in the genus *Hemileia* because of the shape and structure of the urediospores. They did not observe teliospores. *Hemileia* is excluded from consideration, however, because the sori do not emerge from the stomata as fascicles of hyphae. The hyaline basal cells are readily observable and each bears ten or more spores, usually apically and along one side. Frequently the basal cells are curved, with the spores produced along the upper convex side.

Scopella bauhiniicola (P. Henn.) comb. nov. (figs. 5, 6). (*Uredo bauhiniicola* P. Henn., Hedwigia **34**: 98. 1895.)

Telia hypophyllous in the old uredial infections, waxy, yellowish, pulvinate, 0.1–0.3 mm. in diameter or becoming confluent, subepidermal; teliospores borne in groups of 5–7 or more on short, clavate, laterally free basal cells, ellipsoid, ovoid or oblong-ellipsoid, 13–18 × 30–45μ, contents yellowish; wall hyaline, smooth, uniformly 1–1.5μ thick, without a germ pore; pedicels once and one-half to twice as long as the spore, with a central persistent strand and an outer hygroscopic portion which swells to broader than the spore and may ultimately dissolve, hyaline. The teliospores germinate at once by the elongation of the apex of the spore.

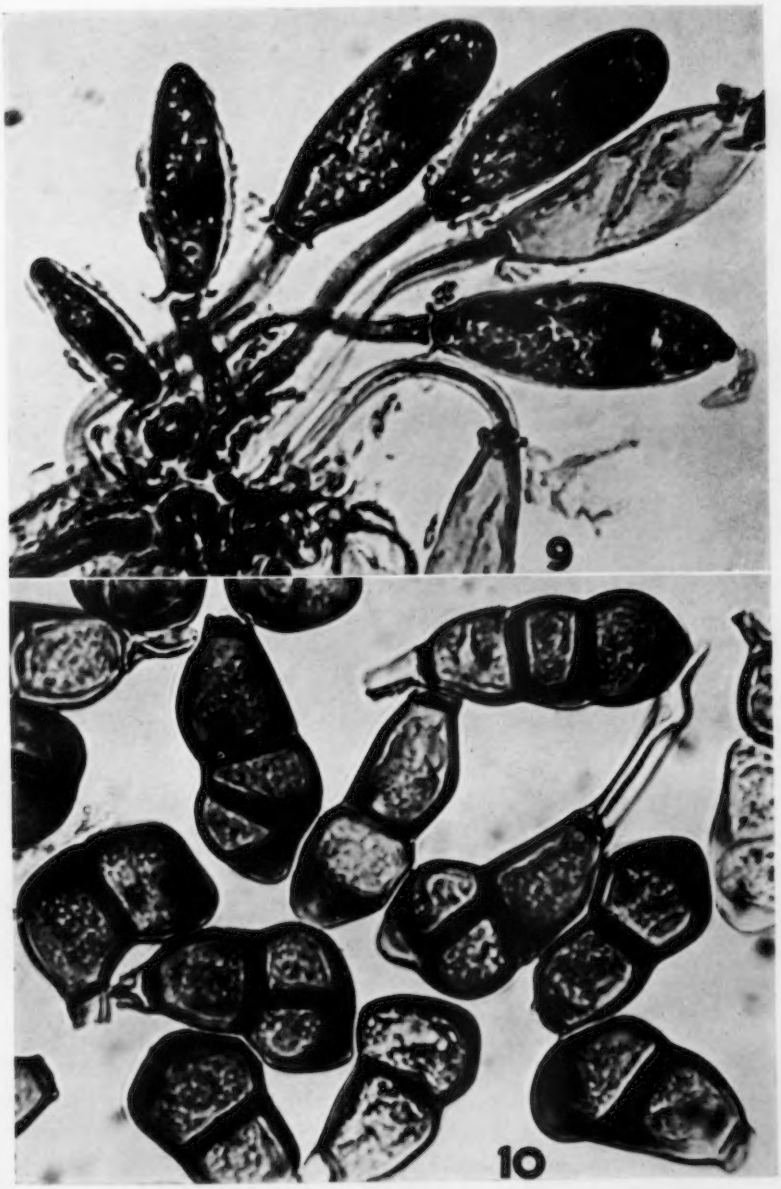

Fig. 9. Photograph of a group of teliospores of *Scopella lucumae* (stained). The outer portion of the pedicel being hygroscopic swells and dissolves leaving only a slight collar next the spore. ×800.

Fig. 10. Photograph of teliospores of *Puccinia poikilospora*. In addition to the two- and three-celled spores, four-celled spores with the same conformations also occur commonly. ×750.

The telia described here were found in old uredial infections on *Bauhinia heterophylla* Kunth, collected March 25, 1919 at Canasi, Prov. Matanzas, Cuba, by J. R. Johnston.

This interesting species is especially distinctive because of the swollen pedicels of the teliospores. After soaking in water the outer portion appears to dissolve, or at least becomes invisible, while the central strand maintains the connection between the spore and the basal cell. The basal cells are free in their upper portion and attach below to a large-celled tissue three or more cells in thickness. Hyphae of large diameter (4–6μ) branch off from the innermost boundary of the cellular layer and are easily observed in the tissue of the leaf.

During my study of *S. bauhiniicola* I examined a specimen on *Lucuma cainito*, vicinity of Para, Brazil, May 15, 1908, *C. F. Baker*. This rust was determined by Dietel as *Uromyces lucumae* Diet., and what is probably the same collection was issued in Sydow Fungi exot. exs. *6*. The teliospores (fig. 9) of this rust are strikingly similar to those of *S. bauhiniicola* in size 14–19 $\times$ 38–54μ) and also have hygroscopic pedicels whose outer portion dissolves away. Usually the only indication of the nature of this outer portion is a persistent collar-like remnant attached to the spore. The persistent central strand resembles that described for *S. bauhiniicola*.

Unfortunately, I have been unable to observe satisfactorily the basal attachment of the pedicels of *Uromyces lucumae*. Several spores may attach to an irregular and rather large basal cell but there seems little evidence that the basal cells are elongate or laterally free and they may be components of a cellular hymenium. If this is true the cells of the hymenium are larger than in other species with which I am acquainted.

The taxonomic status of *U. lucumae* is open to question. If the basal cells can be demonstrated to be laterally free it belongs in the genus *Scopella* but if the basal cells are in reality components of a compact cellular hymenium it belongs in the genus *Maravalia*. Basal cells, as other morphological structures, are presumably subject to considerable variation. There is no rule by which one can definitely decide when basal cells cease to be basal cells and become parts of a cellular hymenium. This question does not arise in connection with the highly developed basal cell of *Scopella echinulata* (Niesel) *Mains*. It begins to enter when one studies *S. cryptostegiae* and *S. bauhiniicola*, however. Knowledge of the pycnia and aecia of these species would help to clarify the situation.

Because of the marked similarity between *Scopella bauhiniicola* and *Uromyces lucumae* Diet. the latter is here transferred to the genus *Scopella* as **Scopella lucumae** (Diet.) comb. nov. (fig. 9).

Uredo wakensis sp. nov.

Uredia amphigena, subepidermalia, dense aggregata in maculis 1–3 mm. diam., pulverulenta, cinnamomeo-brunnea; urediosporae obovoideae vel globoideae, 19–23 × 21–26μ; membrana 1.5–2μ cr., cinnamomeo-brunnea, moderate echinulata, poris germ. 2, aequatorialibus.

On *Tournefortia* sp., Wake Island (taken at Honolulu, Hawaii, Feb. 22, 1938 by A. M. Mito as number 12603. Communicated by Dr. J. A. Stevenson). Type specimen in the Arthur Herbarium, Purdue University Agricultural Experiment Station and in the Mycological Collections of the Bureau of Plant Industry, United States Department of Agriculture.

The closely aggregated uredia occupy slightly hypertrophied spots and have the appearance of uredinoid aecia but no pycnia were found by sectioning.

PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION

Conidial Germination of the Cotton Root-rot Fungus *

Elizabeth Ojerholm Roberts

The conidia of the root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, which are produced in large numbers on the soil surface, have been the object of studies by Duggar, Taubenhaus and Ezekiel, Peltier and King, and others. Spore cultures would extend our knowledge of the life history of the fungus and offer some promise of the development of control measures. Germination under various culture conditions has been reported to occur sparingly or not at all, and the growth resulting from germination to be limited and transitory.

Fresh conidial mats were collected from various localities in Texas and Arizona and used immediately or stored under one of the following conditions: 5°C for three days or more, room temperature for varying periods, room temperature followed by three days or more at 5°C. For testing, fresh or stored conidia were treated with mercuric chloride solution and placed in plate or hanging drop cells for germination counts. Various decoctions and synthetic media were used at different temperatures, light intensities, and atmospheric compositions, and in the presence of living cotton tissue.

Maximum germination of conidia, which never exceeded 5 per cent, occurred when they were incubated at a temperature of 30°C in darkness for three days after the fresh material had been stored at a temperature of 5°C. Spores that had been stored at room temperature for several months and failed to germinate developed germ tubes after storage at 5°C. It is obvious from this that dormancy rather than non-viability may account for the failure of the spores to germinate. Germination took place at 30°C in the following media: egg albumen agar, potato dextrose agar, Weindling's agar, potato dextrose and manure decoction broth, potato dextrose agar + yeast extract, potato dextrose agar + manure. These media were equally favorable for spore germination. Germ tubes frequently reached a length of 34 microns, or five to seven spore diameters, considerably longer than those figured by Taubenhaus. The duration of the germ tube averaged three days.

Other incubation conditions and other media showed reduced or no germination. The addition of 10 per cent carbon dioxide to the atmosphere checked all germination. No germination was obtained on silica gel to which was added nutrients or on black soil mixed with a solution of glucose

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and K_2HPO_4. After passage through the alimentary tract of earthworms, spores failed to germinate. Spores did not grow in decoctions of bind weed, leaf mold, soil, cotton root, and in Waksman's agar, and in agar + 5 per cent sorghum molasses. Extract media prepared from ground yeast and filtered broth cultures of yeast, *Fusarium sp.* or *Trichoderma lignorum* did not induce spore germination. Attempts to induce germination of the spores on living tissue of cotton, bind weed, potato, and carrots failed. Volatile substances given off from the cotton plant tissue had no effect on germination. Germination was not improved by contact with *Phymatotrichum* hyphae according to the method of Ferguson.

The failure of the germinated conidia to continue development may be explained by the assumption that the fungus is heterothallic and that cultures so far tried contain but one of the necessary plus and minus strains. Mixed cultures were seeded with conidia from different localities without prolonging the period of growth or resulting in anastomosis.

The conidia of the cotton root-rot fungus may remain viable for considerable periods of storage at room temperature and be induced to germinate by appropriate treatment. The low percentage germination observed is doubtless in part due to the treatment with mercuric chloride solution. Although the development of the germ tube is extended by incubation at 30°C it is strictly limited. Whether exacting culture requirements or failure to secure mixed cultures of heterothallic strains is responsible for this has not yet been determined. The growth of the germ tube furnishes an identification check, since growth of Phymatotrichum is limited and that of contaminants is not.

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